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## SECTION B—VOL XXV

	PAGE
<b>No 1—January, 1947</b>	
On the Metamorphosis of Two Leptocephali from the Madras Plankton	
<i>R Velappan Nair</i>	1
Studies in the Genus <i>Colletotrichum</i> —III	
<i>T S Ramakrishnan</i>	15
Additions to Fungi of Madras—I	
<i>T S Ramakrishnan and K Ramakrishnan</i>	28
A New Rust on <i>Premna tomentosa</i> Willd	
<i>T S Ramakrishnan and C K Soumini</i>	35
<b>No 2—February, 1947</b>	
Fruit Rot of Tomatoes caused by <i>Phytophthora palmivora</i> Butl	
<i>T S Ramakrishnan and C K Soumini</i>	39
<i>Caligus scaenae</i> N Sp Parasitic on <i>Sciaena glauca</i> from Madras	
<i>C P Gnanamuthu</i>	43
<b>No 3—March, 1947</b>	
(I) The Interaction between Ions Drugs and Electrical Stimulation as Indicated by the Contraction of Avian Unstriated Muscle (II) Active Elongation of Unstriated Muscle	
<i>Inderjit Singh Mrs Sumita Inderjit Singh and M C Muthana</i>	51
Contributions to the Bionomics Anatomy Reproduction and Development of the Indian House Gecko <i>Hemidactylus flaviviridis</i> Ruppel Part IV	
<i>The Respiratory and Vocal Organs Beni Charan Mahendra</i>	57
<b>No 4—April, 1947</b>	
On Three Coccidian Parasites <i>Wenyonella mackinnoni</i> n sp <i>Eimeria lucknowensis</i> n sp and <i>Isospora</i> sp from the Intestine of the Wagtail <i>Motacilla alba</i> Linn (Passeriformes Motacillidae)	
<i>P L Misra</i>	75
Studies on the Refractive Index of Milk Part I Observation on Genuine Samples	
<i>K S Rangappa</i>	86
<b>No 5—May, 1947</b>	
On <i>Beantopsis rajmahalensis</i> gen et sp nov A New Type of Gymnosperm Female Fructifications from the Jurassic of Behar	
<i>P N Ganju</i>	95
<i>Onitheimithus polyandra</i> gen et sp nov A New Type of Fossil Gymnosperm Male Fructifications from the Rajmahal Hills	
<i>P N Ganju</i>	105

<i>Ontheostrobus sessilis</i> gen. et sp. nov. A New Type of Seed Bearing Gymnosperm Fructifications from the Jurassic of Onthea in the Rajmahal Hills	<i>P. N. Ganju</i>	119
Symposium on Statistical Methods in Plant and Animal Breeding		126
Specificity of Bacterial Symbiosis in Aphrophorine	<i>S. Mahdihassan</i>	155

# No. 6—June, 1947

The Mode of Action of Nerves on Unstriated Muscle	<i>Inderjit Singh and Mrs. Sunita Inderjit Singh</i>	163
Influence of Root Excretions and Germinating Seeds on Nitrogen Fixation by <i>Azotobacter</i>	<i>B. N. Uppal, J. A. Daji and M. K. Patel</i>	173
Additions to Fungi of Madras—II	<i>T. S. Ramakrishnan and K. Ramakrishnan</i>	178
Two Species of Undescribed Lampyrid Larvae from S. India	<i>J. Samuel Raj</i>	188

# ON THE METAMORPHOSIS OF TWO LEPTOCEPHALI FROM THE MADRAS PLANKTON\*

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CONTENTS		PAGE
1. INTRODUCTION .. .. .		1
2. METAMORPHOSIS OF THE LEPTOCEPHALUS OF <i>Muraenesox cinereus</i> (FORSKÅL)—STAGES I TO VI .. ..		2
3. METAMORPHOSIS OF THE LEPTOCEPHALUS OF <i>Muraena macrura</i> BLEEKER—STAGES I TO V .. ..		8
4. GENERAL REMARKS .. .. .		12
5. ACKNOWLEDGEMENT .. .. .		13
6. REFERENCES .. .. .		13
7. EXPLANATION OF PHOTOGRAPHS .. .. .		14

## INTRODUCTION

A LARGE number of Leptocephali have been described from different parts of the World, but correlation by actual observation on the metamorphosis as done by Grassi and Calandruccio (1896) has been achieved only in a few species. Lea (1913) and Fish (1927) mention about 16 species of eels of which the larval stages are known, most of them being correlated by myotome and vertebral counts, a character which remains constant throughout the life-history of the eels.

The first record of Indian *Leptocephali* appears to be that made by Kaup (1856), who gave an account of *Leptocephalus acuticaudatus* and *Leptocephalus dussumieri* collected from Malabar, *Leptocephalus marginatus* from Pondicherry and *Leptocephalus taenia* from India. Later, Southwell and Prashad (1919) described *Leptocephalus milnei* and *Leptocephalus vermicularis* obtained from the brackish waters of the Gangetic Delta. Apparently the latter can only be an advanced elver stage of the former, since the myotome numbers of the two are closely similar. A preliminary study of the eel eggs and larvae of the Madras Coast was made by Aiyar, Unny and Varkey (1944, Abstract). Recently, Govinath (1946) has

\* Work carried out under a scheme of research financed by the Imperial Council of Agricultural Research, New Delhi.

recorded the occurrence of the *Leptocephalus* and elver stages of *Congrellus anago* along the Trivandrum Coast

It may be mentioned here that Cantor (1850) described *Leptocephalus dentex* found in a partially digested condition in the stomach of *Johnius diacanthus* at Pinang Kaup also recorded *Leptocephalus taenia* from the Maldives Deraniyagala (1934) described some apodal larvae collected from Ceylon waters

This being our state of knowledge of the Indian eels and their larvae the present investigation is the first attempt to correlate the *Leptocephali* occurring along the Madras Coast with the adult eels by allowing them to metamorphose in the Laboratory So far two types of eel larvae have been noted to occur commonly in the Madras Plankton during the months January to April The occurrence of swarms of the *Leptocephali* of *Muraenesox cinereus* and *Muraena macrura* in the living condition in the Plankton collections made on the 11th April 1945 and 14th February 1945 respectively gave a unique opportunity to study the changes undergone by them during metamorphosis The young eels thus metamorphosed lived in a healthy condition in the Laboratory tanks for about 2½ and 4 months respectively Based on the characters of the metamorphosed young eels it is possible to say that one type is *Muraenesox cinereus* of the Family Congridæ characterised by the presence of canine teeth in the front parts of the jaws and on the vomer and that the other type is *Muraena macrura* of the Family Muraenidæ distinguished by the origin of the well-developed dorsal fin before the gill opening and the ventral fin immediately behind the anus

#### METAMORPHOSIS OF THE LEPTOCEPHALUS OF *Muraenesox cinereus* (FORSKÅL)

##### Stage I †(Fig 1 and Photographs 1 and 2)

	mm
Total Length	81
Length of Head	5
Length of Trunk	48
Length from Anus to tip of Tail	28
Length from tip of Snout to origin of Dorsal Fin	20
Maximum Height including Vertical Fins	11
Maximum Height excluding Vertical Fins	10
Total Myotome number	138
Anal opening below Myotome	78

† The stages described are arbitrary and the measurements and the descriptions given are those of the different stages shown in the photographs

*On Metamorphosis of Two Leptocephals from Madras Plankton* 3

The *Leptocephalus* is completely transparent with the body strongly compressed moderately elongate tapering towards the head and the tail particularly towards the latter. This is partly due to the presence of long caudal fin rays which are approximately double the size of the rays of the other vertical fins. Head is elongated with a sharply ending snout. Both the jaws are of equal length and carry teeth which are small pointed and directed forwards. The cleft of the mouth is straight horizontal and extends to about the same level as the posterior edge of the eye. The alimentary canal is straight and the anus opens to the outside below the 78th myotome. The pectoral fin is small with faint indications of the rays. The following coloration is quite characteristic. Three to four stellate black chromatophores are present on the middle of the sides of the upper jaw placed at regular intervals. In the heart region similar chromatophores numbering four to five are present. In the region of the entire length of the alimentary canal and at the bases of the anal and caudal fins chromatophores are present with no regularity in their arrangement. The dorsal and the pectoral fins are devoid of any chromatophores. An extensively branched chromatophore is present in the middle region of each myocomma from the 17th myotome onwards. These are regularly arranged in a line just below the level of the vertebral column. In the preceding myocommas there are only three to four chromatophores which though not regular in their arrangement are in a line with the others.

*Stage II* (Fig 2 and Photograph 2)

	mm
Total Length	73
Length of Head	6
Length of Trunk	25
Length from Anus to tip of Tail	42
Length from tip of Snout to origin of Dorsal Fin	11.5
Maximum Height including Vertical Fins	8
Maximum Height excluding Vertical Fins	6.5
Anal opening below Myotome	50

In this stage certain changes have taken place indicating that the larva has begun to metamorphose. The larva in this stage is very active in its movements and is slightly opaque and cannot be regarded as completely transparent. Of the noteworthy changes the diminution of the height of the larva is very striking. Consequent on this change the dorsal and the ventral fins have become slightly wider and prominent. There is also a proportionate increase in the width of the larva. Changes affecting the shape of the head have commenced the snout having become very blunt the

larval teeth have begun to fall off. The anus has shifted forward considerably and occupies a position below the 50th myotome. The blood in this stage is almost colourless except for a slight red patch in the vicinity of the heart. There is also a general increase in pigmentation. In the anterior part of the snout a group of irregular chromatophores has made its appearance. An increase in the number of chromatophores is noted in this stage throughout the length of the alimentary canal. These chromatophores are more concentrated on the dorsal side of the alimentary canal than on the ventral side where they are comparatively few in number and are widely scattered. Along the bases of each of the rays of the anal fin chromatophores have appeared in a row. In addition to these, a row of eight to ten chromatophores is present in the anterior portion of the anal fin at the region of demarcation of the fin from the body. The borders of the dorsal and anal fins contain a few widely scattered irregularly arranged chromatophores. On the body, though there is no increase in the number of chromatophores, those that are present on the myocommas of the larva are slightly larger and more prominent than the others.

*Stage III.* (Fig. 3 and Photograph 2).

Total Length	..	..	..	..	67
Length of Head	.	..		.	6.5
Length of Trunk	..	..	..	..	18.5
Length from Anus to tip of Tail			.	..	42
Length from tip of Snout to origin of Dorsal Fin					10
Maximum Height including Vertical Fins				.	7.5
Maximum Height excluding Vertical Fins					5
Anal opening below Myotome			..	..	44

Remarkable changes in the shape of the head have taken place in this stage. The height of the body has decreased considerably with a proportionate increase in the width. The blood has assumed the bright red colour and the larva has ceased to be transparent. The larval teeth in this stage have completely dropped out. This and the subsequent stages are edentulous and the larvæ do not feed during metamorphosis. More chromatophores are added in the region of the snout. A few chromatophores are present in the anterior region of the lower jaw and in the region behind the eyes. Pigment cells have begun to appear on the dorsal half of the body with a concentration at the base of the dorsal fin. On the margins of the dorsal and the anal fins small chromatophores are present giving an indication of their coloration in the adult condition.

*Stage IV* (Fig 4 and Photograph 2)

	mm.
Total Length	68
Length of Head	6 5
Length of Trunk	20 5
Length from Anus to tip of Tail	41
Length from tip of Snout to origin of Dorsal Fin	9
Maximum Height including Vertical Fins	7
Maximum Height excluding Vertical Fins	4 5
Anal opening below Myotome	43

The shape of the head has changed considerably the blunt snout giving the appearance of an adult eel. The height of the body has decreased further with an increase in width. The body has become completely opaque and white in colour. The larva in this stage is very active and swims about in the aquarium with great rapidity. The anus has shifted still further and is under the 43rd myotome. The pigmentation is more pronounced in this stage. The tip of the snout is dark due to the accumulation of chromatophores. Similarly on the tip of the lower jaw chromatophores have begun to concentrate. Groups of chromatophores are present on the dorsal side of the head and behind the eyes. There is an intensification of the uniformly spread chromatophores in this stage on the dorsal side of the body at the base of the dorsal fin and these chromatophores on the sides are arranged along the myocommas only. A few scattered chromatophores are also present on the myotomes. Numerous chromatophores have formed on the edges of the dorsal and anal fins giving a shaded appearance to the borders of these fins. A few chromatophores are found amidst the caudal fin rays.

*Stage V* (Fig 5 and Photograph 2)

	mm
Total Length	61
Length of Head	7
Length of Trunk	14
Length from Anus to tip of Tail	40
Length from tip of Snout to origin of Dorsal Fin	7 5
Maximum Height including Vertical Fins	6
Maximum Height excluding Vertical Fins	3

In this stage the metamorphosis is almost complete and the adult characters predominate except for the colour. An indication of the adult coloration is discernible even in this stage. The movement of the animal in a serpentine manner and darting away at the slightest disturbance is a

very much like the adult eel. The body is muscular and more or less cylindrical in shape. The adult set of teeth has formed but they are very minute in size. The coloration is prominent owing to the new chromatophores

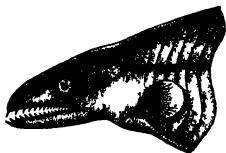


FIG 1

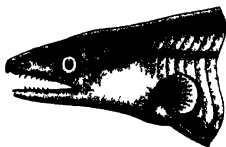


FIG 2

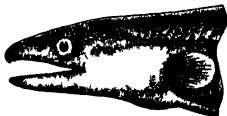


FIG 3



FIG 4



FIG 5



FIG 6

FIGS 1-6 Head region of the six stages in the metamorphosis of the *Leptocottalus armatus*  $\times$  *C. 7*

which have appeared and by the spreading of the chromatophores already present. The coloration is brighter on the dorsal side of the head and the body and also on the margins of the vertical fins and the caudal fin. On the ventral half of the body also pigment cells have appeared particularly along the myocommas. This is more pronounced on the posterior half of the body. The abdomen is free from chromatophores. This pigmentation is very light when compared with that of the dorsal half of the body. A narrow band without any pigment runs throughout the length of the animal along the lateral line. This band gradually disappears in the tail region.

*On Metamorphosis of Two Leptocephals from Madras Plankton* 7

*Stage VI* (Fig 6 and Photograph 2)

	mm
Total Length	62
Length of Head	8.5
Length of Trunk	12.5
Length from Anus to tip of Tail	41
Length from tip of Snout to origin of Dorsal Fin	8.5
Maximum Height including Vertical Fins	4.5
Maximum Height excluding Vertical Fins	2.75

This stage could be considered as the juvenile eel. Most of the chromatophores of the dorsal side of the head and the body have enlarged and fused with one another with the result that the young eel is uniformly pigmented when viewed from the top, being brownish black with the ventral portion of the head and the abdomen almost free from pigments. The lateral line is indicated by a thin unpigmented streak. The adult set of teeth is prominent.

The young eels were fed on the flesh of prawn and they grew rapidly. One eel lived in the Laboratory tank for about  $2\frac{1}{2}$  months and its rate of growth is as follows —

1 month old eel	120 mm in total length
2 month old eel	160 mm in total length
$2\frac{1}{2}$ month old eel	180 mm in total length

In the last stage the characteristic silvery coloration has begun to appear particularly on the abdomen of the eel.

The important changes that take place in the larva of *Muraenesox cinereus* during metamorphosis may be summarised as follows —

The loss of the transparency of the larva. The loss of the larval set of teeth at the commencement of metamorphosis. The formation of the adult set of teeth after the completion of metamorphosis. The gradual assumption of the adult coloration by the formation of stellate brownish black chromatophores. The gradual acquisition of the red colour of the blood. The shifting of the anus and the origin of the dorsal fin to an anterior position. The decrease of the body height with the vertical fins becoming more prominent and with a proportionate increase in the width of the animal. The changes affecting the shape of the head.

It is interesting to mention here that these changes resulting in the complete metamorphosis are undergone in the short time of about ten days,

R. Velappan Nair

METAMORPHOSIS OF THE LEPTOCEPHALUS OF *Muraena macrura* BLEEKER

Stage I (Fig 7 and Photograph 3)

	mm
Total Length	93
Length of Head	4
Length of Trunk	58
Length from Anus to tip of Tail	31
Length from tip of Snout to origin of Dorsal Fin	50
Maximum Height including Vertical Fins	13
Maximum Height excluding Vertical Fins	12.5
Total Myotome Number	216
Anal opening below Myotome	120

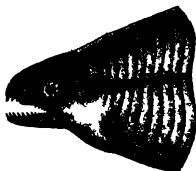


FIG 7

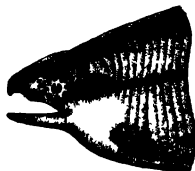


FIG 8

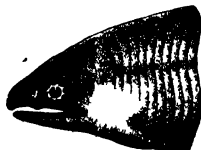


FIG 9



FIG 10



FIG 11

FIGS 7-11 Head region of the five stages in the metamorphosis of the Leptocephalus of *Muraena macrura*,  $\times Cq 7$

*On Metamorphosis of Two Leptocephali from Madras Plankton* 9

The larva of *Muraena macrura* can be easily distinguished from that of *Muraenesox cinereus* by certain characteristic features. The larvae are completely transparent and are very slow in their movements. It is not quite easy to locate them in water and the only sign to detect them is the prominent colour of the eyes. When disturbed they roll sideways to form a spiral with the head in the centre which may be another device for protection in addition to transparency. Unlike the other Leptocephali they seldom make an attempt to swim away.

The body is remarkably thin and high and has a leafy appearance. The two extremities are quite blunt and especially so in the caudal region. The head is roughly conical with a blunt snout. The lower jaw is pointed and is of about the same length as the upper one. Both jaws carry strongly pointed teeth which are directed forwards. The cleat of the mouth is straight and extends to about the centre of the eye. The alimentary canal has a straight course and opens to the outside below the 120th myotome. Though the adults are without the pectoral fins they are present in the larval stages very near the gill opening. The coloration is constant in this species particularly in the region of the head. The eyes are coloured golden yellow and round the central circular black region are placed eight large stellate black chromatophores of uniform size. These chromatophores are regularly arranged and are placed equidistant from one another. The golden yellow coloration is also present as patches in the anterior and posterior regions adjacent to the eye. The posterior patch is larger than the anterior one. Black stellate chromatophores are present on the middle dorsal region of the alimentary canal. At the bases of the anal fin, the caudal fin and the posterior third of the dorsal fin similar chromatophores are present arranged in a line. These chromatophores are not conspicuous in the living condition but could be made out only in the preserved condition with the aid of a magnifier.

*Stage II* (Fig 8 and Photograph 3)

	mm
Total Length	77
Length of Head	5
Length of Trunk	48
Length from Anus to tip of Tail	24
Length from tip of Snout to origin of Dorsal Fin	40
Maximum Height including Vertical Fins	11.5
Maximum Height excluding Vertical Fins	11
Anal opening below Myotome	120

This stage shows the commencement of the metamorphosis of the larva. The larval teeth are shed completely and the larvae remain edentulous for

the duration of metamorphosis and apparently without taking any food. The pectoral fins have begun to show signs of atrophy and in this stage they are considerably smaller than those of the preceding stage. The only other marked change is the reduction in the height of the larva. There is no shortening of the alimentary canal in this stage and the anus is under the same myotome as before. But for the appearance of three to four small chromatophores in the region of the snout and a slight opacity the coloration of the larva remains unchanged.

*Stage III* (Fig 9 and Photograph 3)

	mm.
Total Length	80
Length of Head	5.5
Length of Trunk	47.5
Length from Anus to tip of Tail	27
Length from tip of Snout to origin of Dorsal Fin	40
Maximum Height including Vertical Fins	8.5
Maximum Height excluding Vertical Fins	8
Anal opening below Myotome	120

Many changes have taken place in this stage of which those affecting the shape of the head are especially noted. The jaws, particularly the upper one, have elongated and become more pointed. The cleft of the mouth has increased in length and extends to about the posterior border of the eye. The height of the body has decreased considerably. There is no anterior shifting of the anus and its position is the same as in the previous stages. The blood is faintly red and the larvae are more or less opaque. They are more active than before and the slightest disturbance makes them swim rapidly in the aquarium. The rolling habit of the larval stage is no longer observed. Pigmentation remains unchanged except for the appearance of a few more chromatophores in the upper jaw and a few pigment cells in the lower jaw.

*Stage IV* (Fig 10 and Photograph 3)

	mm
Total Length	78.5
Length of Head	6.5
Length of Trunk	40
Length from Anus to tip of Tail	32
Length from tip of Snout to origin of Dorsal Fin	30
Maximum Height including Vertical Fins	6.5
Maximum Height excluding Vertical Fins	6
Anal opening below Myotome	103

*On Metamorphosis of Two Leptocephali from Madras Plankton* 11

The height of the body has diminished to a marked extent with a proportionate increase in the width of the animal. The head has almost assumed the shape of the adult eel with the cleft of the mouth extending beyond the posterior limit of the eye. The prominent pigmentation of the eyes has begun to disappear due to the gradual darkening of the peripheral region. The pectoral fins are represented in this stage as mere vestiges near the border of the gill opening. Only from this stage the anus begins to move forward to occupy an anterior position being situated in this stage under myotome 103. The blood is brightly coloured and the vicinity of the heart is bright red. The transparency of the larva is completely lost the metamorphosing one being perfectly opaque. The larvae are very active in their movements. Brown chromatophores have appeared uniformly all over the head and the body with a concentration on the head and the vertical fins thus giving an indication of the adult coloration.

*Stage V* (Fig 11 and Photograph 3)

	mm
Total Length	69
Length of Head	9
Length of Trunk	23
Length from Anus to tip of Tail	37
Length from tip of Snout to origin of Dorsal Fin	8
Maximum Height including Vertical Fins	4.5
Maximum Height excluding Vertical Fins	3

This is the final stage in the metamorphosis of the larva where the adult characters are assumed in all respects and the typical appearance of the *Muraenid* eel is reached even in regard to coloration. The head has transformed completely with the cleft of the mouth extending posterior to the eye to about an equal distance as the length of the snout. The larval coloration of the eyes is completely lost and they are dark in colour. The adult set of teeth has appeared. The pectoral fins are completely lost and no trace of them could be seen in this stage. The body is perfectly cylindrical due to the great reduction in the height of the larva. The anus has shifted still anteriorly over a considerable distance. The vertical fins have become very broad and prominent. The young eel is uniformly brownish black due to the presence of numerous closely set stellate chromatophores.

These also feed voraciously on the flesh of prawns. Growth is rapid and the rate in one specimen is as follows,—

1 month old eel	..	..	120 mm. in total length.
2 month old eel	..	..	160 mm. in total length.
3 month old eel	..	..	200 mm. in total length.
4 month old eel	..	..	240 mm. in total length.

As they grow the young eels become dark brownish black in colour (Photograph 4).

Reviewing the changes undergone during the metamorphosis of this Muraenid larva into the adult eel, we find that these are essentially the same as those of *Muraenesox cinereus* with some characteristic differences. The noteworthy changes are:—

The loss of the transparency of the larva. The loss of the larval set of teeth at the commencement of metamorphosis and the edentulous condition of the larva till the assumption of the adult characters. The gradual atrophy and the complete loss of the pectoral fins. The gradual assumption of the red colour of the blood. The shifting of the origin of the dorsal fin to an anterior position and the elongation of the head. The decrease of the body height to a considerable extent leading to the cylindrical shape of the eel. Unlike *Muraenesox cinereus*, the adult pigmentation is not observed in the first three stages but when the larva reaches the fourth stage the adult pattern is rapidly formed. The same is observed with regard to the anterior shifting of the anus which remains in the same position in the first three stages. The anus moves forward over a considerable distance during the last two stages. The vertical fins become wide and prominent only in the last stage of metamorphosis. The time taken for complete metamorphosis is about ten days which is about the same as that taken by *Muraenesox cinereus*.

#### GENERAL REMARKS

It is well known that the European eel, *Anguilla vulgaris* and the American eel, *Anguilla rostrata*, migrate to the common breeding place in the Western Atlantic and that their larvae, *Leptocephalus brevirostris* and *Leptocephalus grassi*, have three and one years of pelagic larval life respectively before they reach their respective Coasts. Very little is known about the breeding places of the Indian eels. Deraniyagala (1929) has collected the elvers of *Anguilla bicolor* and *Anguilla elphinstonei* from the Pearl Banks and suggests (1934) the possibility of Ceylon Anguillids reproducing throughout the year in the Coastal waters. According to Schmidt (Deraniyagala, 1934) the sea west of Sumatra is the breeding ground of eels and from here he has collected the tiny larvae of *Anguilla bicolor* and *Anguilla elphinstonei*. He considers that the larvae approach the Coast only at the end of their

*On Metamorphosis of Two Leptocephali from Madras Plankton* 13

pelagic life Rahimullah Mahmood and Kabir (1944) are of opinion that *Anguilla bengalensis* breed in freshwater leaving its catadromous breeding habits

From the Madras Plankton only the final pelagic larval stages of the two Leptocephali studied have so far been collected though regular collections have been made during the past ten years \* If the eels reproduce throughout the year in the Coastal waters as suggested by Deraniyagala it should be possible to get at least a few of the earlier stages of the larvæ especially when they occur in enormous numbers This seems to show that the eels do not breed near the Madras Coast and that probably their breeding place is the open sea

Grassi and Calandruccio (1896) have shown that *Leptocephalus brevirostris* takes about a month for the transformation into the elvers in the aquarium at Naples The metamorphosis is much quicker in the two forms studied at Madras as they take only ten days to complete the metamorphosis This is to be expected owing to the higher temperature conditions in which these Leptocephali grew and metamorphosed

#### ACKNOWLEDGEMENT

My thanks are due to Dr N Kesava Panikkar who was formerly the Director of the University Zoological Research Laboratory Madras for valuable help and suggestions

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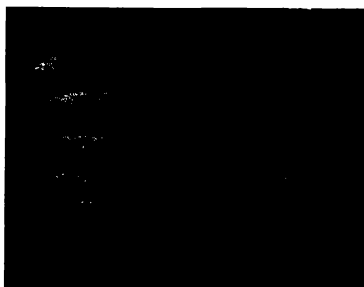
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### EXPLANATION OF PHOTOGRAPHS

- 1 A small portion of the swarm of the *Leptocephalus* of *Muraenox cinereus* collected on the 11th April 1945  $\times$  Ca 3/10
- 2 The six stages in the metamorphosis of the *Leptocephalus* of *Muraenox cinereus*  
About nat size
- 3 The five stages in the metamorphosis of the *Leptocephalus* of *Muraena macrura*  
About nat size
- 4 Four month old *Muraena macrura*  $\times$  Ca 3/8



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## STUDIES IN THE GENUS COLLETOTRICHUM—III

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- IN earlier communications (Ramakrishnan, 1941)<sup>1,2</sup> the parasitism of *Colletotrichum indicum* and the occurrence of saltations in *C. capsici* Syd. have been dealt with. During the course of further investigations it was observed that a close resemblance existed between these two species and some other isolates belonging to this genus. The results of these investigations embodying the studies of certain aspects of the physiology of *C. indicum* and the comparison of the isolates from *Capiscum annuum* (*C. capsici*), *Curcuma longa* Syd. (*C. curcuma* Syd.), *Aristolochia bracteata* Retz. and *Cicer arietinum* (*C. capsici*) are recorded in this paper.

### MATERIALS AND METHODS

The isolate of *C. indicum* Dast. obtained from specimens sent by Prof. Dastur in 1938 was used. *C. capsici* was isolated from diseased specimens of *Capiscum annuum* from the Agricultural Research Station, Taliparamba (Malabar). Sundararaman (1930) and Thomas (1941) have recorded the occurrence of *Colletotrichum* on *Aristolochia bracteata* in Coimbatore. The fungus was isolated from fresh leaf-spots on this host. Fresh specimens of diseased leaves of *Curcuma longa* were obtained from Bhavani (Coimbatore District) through the courtesy of Sri. A. Rathnavelu, the Agricultural Demonstrator, Bhavani, and the fungus *C. curcuma* was obtained from these. *Cicer arietinum* was affected by a blight at Pollachi (Coimbatore). This was found to be due to *Colletotrichum* and this isolate was also included in the comparative studies. Sundararaman (1926) has recorded *Colletotrichum* (*Vermicularia*) on *Cicer arietinum*.

The cultures of the isolates were initiated from single spores and maintained on either oat agar or french bean agar. Petri-dish cultures were grown inside an incubator at 32° C. unless otherwise stated. The dry weights of the fungus growths and the reaction of the fungus to different sources of nitrogen and carbon were determined following the method described by Ramakrishnan (1941).<sup>3</sup> For all inoculation experiments on cotton *G. herbaceum* strain H<sub>1</sub> was employed.

*A. Physiology of C. indicum.*

*Temperature relations.*—The isolate grows well on agar media at the laboratory temperature (27° to 30° C.). The relative growth and sporulation at other temperatures also were studied. The fungus was inoculated into Petri-dishes containing oat agar and french bean agar and the dishes were transferred to controlled temperature chambers where the temperatures were maintained at 5°, 10°, 15°, 20°, 32°, 37° and 44° C. respectively. Closer intervals could not be utilised. The results were as follows:—

TABLE I

Temperature	Oat agar		French bean agar	
	Diameter in mm. in 7 days	Nature of growth	Diameter in mm. in 7 days	Nature of growth
5° C.		No growth		
10° C.				
15° C.	Slight development 31.3	Dark growth, black stromata in the centre, very few acervuli	Not measurable.	Dark, thin growth few acervuli
20° C.	63.5	Olive-black growth, grey aereal mycelium, fair production of acervuli.	28.5	Black centre, lighter outside, numerous acervuli.
32° C.	80.0	Aerial mycelium pale olive grey, numerous buff pink acervuli.	73.7	White and grey aereal growth, numerous black stromata and pink acervuli.
37° C.	62.3	Acervuli fewer.	54.25	Less of aereal growth and fewer acervuli.
44° C.		No growth		

The best growth occurs in the neighbourhood of 32° C. among the temperatures under trial. Sporulation is evident between 15° and 37° C. and the maximum acervular development is at about 32° C. There was no development at 5° C. but the fungus remained quiescent. When the dish was transferred from 5° C. to the laboratory temperature after one week, the fungus began to grow again and covered the dish in 8 days. The dishes kept at 44° C. did not exhibit any growth and even after removal to the laboratory temperature a week later there was no revival of the fungus. Continuous exposure to 44° C. for a week had evidently killed the fungus. Ling and Yang (1941) have found that the Chinese isolate of *C. indicum* grew best at 28° C. This temperature, however, was not included in the experiments conducted here and therefore it cannot be said that the optimum temperature for the local isolate is different. But the same authors have

also found that even in the Chinese isolate the highest germination of spores and the maximum length of germ tubes are at 32° C.

The dry weights of fungus mats grown in liquid cultures at different temperatures were also recorded. The results are given below.

TABLE II

Temperature	Dry weight in milligrammes of fungus mat (17 days' old)
10° C.	43.0
18° C.	243.0
30° C.	245.6
30° 5' C.	265.3
32° C.	315.3
37° C.	165.3

These results show that liquid cultures follow a similar trend as the dish cultures.

Temperature is known to influence the spore size in some fungi (Johann, 1913, Ramakrishnan, 1941, 3). Measurements of spores from the cultures kept at different temperatures were taken and the mean length and frequency distribution are given below.

TABLE III

Class in $\mu$	Frequency distribution at							
	15° C.		20° C.		32° C.		37° C.	
	Oat agar.	F. bean agar.	Oat agar.	F. bean agar.	Oat agar.	F. bean agar.	Oat agar.	F. bean agar.
10-20	3	3	9	3	16	11	19	
21-35	95	102	111	108	111	95	90	
36-50	96	86	77	88	68	91	81	
51-65	6	9	3	4	5	3	1	
Mean length in $\mu$	26.1	26.0	25.5	25.6	25.2	25.6	25.2	

The spore length has been remarkably constant at all temperatures in this isolate.

The optimum temperature for infection of cotton was determined. Cotton seeds soaked for one hour in a spore suspension in distilled water were sown in pots containing sterilized soil. Twenty seeds were sown in each treatment. The pots were kept in chambers with air temperatures at 15°, 20°, 30° and 35° C. respectively. Control pots containing sterilised soil sown with uninfected healthy seeds were also kept. The pots were kept under observation for one week and the following results were obtained.

TABLE IV

Temperature	Inoculated			Control
	No. germinated	No. of seedlings infected	No. germinated	No. of seedlings dead
15° C.	5	1	6	—
20° C.	11	8	12	—
30° C.	14	14	16	—
35° C.	13	4	16	—

Among the temperatures included in the experiment mortality is high at 30° C.

*Carbon and nitrogen sources on growth and sporulation.*—Different sources of carbon and nitrogen are known to influence the growth and sporulation of *Colletotrichum* in different ways. To ascertain whether this isolate also behaves in a similar manner it was grown on media having a basic composition (Ramakrishnan, 1941, 3) to which equivalent weights of different carbohydrates or nitrogenous substances were added.

*Carbohydrates.*—The fungus was grown on solid and liquid media. The average diameter of the growth after 7 days and the average dry weight of the fungus mat in liquid media after 17 days were determined.

TABLE V

*Statement showing the diameter of growth or dry weight in different carbon sources*

Carbon source	Agar media		Liquid media		
	Diameter in mm 7 days	Remarks	pH at start	pH after 17 days	Weight of dry mat in mg.
Sucrose	68.3	Numerous black and light vinaceous fawn acervuli with spore masses	4.4	6.8	266.7
Glucose	68.5	Black sclerotoid bodies and big light vinaceous fawn spore-bearing acervuli.	4.3	7.3	223.3
Maltose	80.3	Black sclerotoid bodies, acervuli less than in sucrose.	4.4	7.3	270.9
Lactose	71.0	Thin growth, black sclerotoid bodies formed, few acervuli.	4.4	6.0	107.6
Starch (soluble)	74.5	Thin white growth with a number of drab masses. Acervuli more than in lactose.	4.6	7.2	186.4

Maltose and sucrose induce good growth but sporulation is best in sucrose.

TABLE VI

Statement showing the growth of the fungus and spore length on different sources of nitrogen

Source of Nitrogen	Diameter of growth in 8 days mm.	Spore length in microns		REMARKS
		Range	Mean	
Peptone ..	80.8	30-36	27.7	Smoke grey growth, numerous pale vinaceous acervuli all over the growth.
Asparagin ..	43.5	30-32	26.8	Pale smoke grey aereal growth margin regular, numerous acervuli and black stromatoid bodies all over the growth.
Potassium nitrate ..	45.5	30-36	27.4	Pale smoke grey aereal growth, numerous black stromatoid bodies acervuli scattered in growth.
Ammonium sulphate ..	19.7	42-38	24.2	Thick growth, margin crenate, and ridged, pale olive grey aereal growth, very few acervuli.
Urea ..	32.5	16-22	23.3	Mealy, pale greyish vinaceous growth, acervuli more than in ammonium sulphate, spore vacuolated.
Potassium nitrate ..	—	No growth.		

Peptone serves as a good source of nitrogen. The growth is slow and sporulation less when ammonium sulphate or urea are used.

**Staling.**—The uniform rate of growth of the fungus for 10 to 12 days on agar media, does not suggest any accumulation of staling products in the early stages of its growth. But in liquid cultures maintained for over three weeks there is evidence of the development of staling products, as no further increases in weight of fungus were obtained. In order to clear this point the fungus was grown on filtrates from cultures 25 days old. The filtrate was mixed with fresh Richards solution in the proportion of 1:1 and autoclaved before use. The control consisted of Richards solution mixed with an equivalent volume of distilled water before autoclaving. The two sets of media were inoculated from the same culture with equal quantities of inoculum. After fifteen days' growth the fungus mat was removed and the dry weight determined. The weights were as follows:—

TABLE VII

Medium used	Average dry weight of fungus mat in mgm.
Filtrate from culture of <i>C. indicus</i> + Richards solution ..	80.8
Richards solution + Distilled water ..	100.7

From the above it is evident that staling products accumulate in cultures over three weeks old and these inhibit the growth of the organism. The presence of these substances was further demonstrated by allowing fresh seedlings of cotton ( $H_2$  strain) to stand with their roots and hypocotyl immersed in the filtrate (filtered through coarse filter-paper) of cultures 25 days old. The controls were kept with the roots immersed in Richard's solution adjusted to the same pH as the filtrate. In 12 hours the seedlings kept in the filtrate wilted while the controls were quite turgid (Plate III, Fig. 4). Ling and Yang (1944) state that they were not able to demonstrate the production of toxic substances. This may be due to the fact that the toxic staling products had not developed in the filtrate from 10-day-old cultures used by them. Under Coimbatore conditions it was observed that the formation of staling products or their accumulation in sufficient quantity becomes evident only in old cultures. Further these authors have been studying an isolate of the fungus prevalent in China. It is quite probable that the Chinese strain and the Indian strain do not behave alike. This view is supported by the observation that the Chinese strain infects two varieties of *G. hirsutum*, viz., Trice and Delfos—while all the isolates studied in Coimbatore including the strain, kindly supplied by Dastur from Nagpur have not been found to be pathogenic on *G. hirsutum* but only on *G. herbaceum* and *G. arboreum*. This fungus has been under observation in South India for over twenty years and all through this period there has been no record of its occurrence on any strain of *G. hirsutum* though Combodia cotton (*G. hirsutum*) is cultivated over a large area in Coimbatore. Thus neither in nature nor by artificial infection was the fungus found to infect *G. hirsutum*. Dastur (1934) who described the fungus from Nagpur has recorded it only on *G. arboreum*. Consequently it is presumed that the Chinese strain behaves differently from the Indian strain of the fungus in some of its physiological reactions.

**Saltation.**—A number of saltants were developed by this isolate on Richards agar and oat agar in the form of sectors or islands (Plate III, Fig. 3). The saltants exhibited differences in the colour and texture of the growths and in the intensity of sporulation. Non-spore-forming saltants were also formed.

**B. Comparative study of *C. indicum* with *C. capsici*, *C. curcuma* and isolates from gram (*Cicer arietinum*) and *Aristolichia bracteata*.**

A comparison of the external morphology of the different isolates under study revealed a very close resemblance to one another. The appearance of the acervuli on the respective hosts was similar. Very often they exhibited

formation in concentric rings. Normally they are black with a well developed stroma which projects outside the host tissue. On the stroma are developed long dark septate setæ mixed with hyaline one-celled conidiophores. Falcate (crescent-shaped) unicellular, hyaline conidia are formed on these conidiophores. When large numbers of spores are formed the spore mass on the acervulus assumes a deep to light pink colour.

The size of the acervulus exhibits a wide variation in the same host, the range of variation being from 45 to 295  $\mu$ . The range of variation exhibited by setæ of any one isolate is very great. The size of the setæ in agar cultures also varies within wide limits.

The conidia of all the isolates of the same age were of the same shape. Measurements were taken of 200 conidia of each. The range of variations and the average measurements agree very closely. The following table represents the measurements of the spores of these isolates as compared to the original measurements obtained by different authors.

TABLE VIII

Species or isolate	Size of spore given by original authors		Size of spores found on host tissue Author's measurements	
	Length $\mu$	Breadth $\mu$	Length $\mu$	Breadth $\mu$
<i>C. indicum</i> ..	15-25 (Dastur)	1.8-4.3	24.80 (18-31)	3.1
<i>C. capsici</i> ..	17-28 (Butler)	2-4	25.3 (19-31)	3.2
<i>C. curcuma</i> ..	18-29 (Sundaraman)	3-5	25.4 (17-31)	3.1
<i>C. on Aristolochia</i> ..	..	..	24.4 (20-30)	3.2
<i>C. on gram.</i> ..	21-34 (Sundaraman)	3-6	24.5 (22-28)	3.1

(Figures within brackets represent the range of measurements.)

From the above table it can be seen that there is no difference in the spore size between the isolates. On the other hand, there is very close agreement.

On agar media the first generation of the isolates exhibits a medium proportion of pale grey to pale olive grey aerial mycelium and numerous acervuli with pink spore masses. When the same isolate is carried through a number of generations the aerial mycelium diminishes in quantity. Slight differences are noticed between the isolates in the colour developed during the later generations but these fall within the normal variability of the same isolate or may be due to the formation of saltants,

In order to determine the host range of these isolates inoculation experiments were conducted on *G. herbaceum*, *Capiscum annuum*, *Cicer arietinum* and *Aristolochia bracteata*. Fifteen inoculations were made in each case on the respective plants and the results recorded after seven days are noted below.

TABLE IX

Statement showing the number of positive infections at the end of seven days

Isolate	Cotton seedlings	Capiscum fruits	Aristolochia leaves	Gram seedlings
<i>C. indicum</i> ..	16	4	13	16
<i>C. caprici</i> ..	—	11	14	16
<i>C. curcuma</i> ..	—	10	13	16
<i>C. from Aristolochia</i> ..	9	6	14	14
<i>C. from gram</i> ..	8	8	10	14

The controls remained healthy in all cases. The isolates from *Capiscum* and *Curcuma longa* do not affect cotton. All the isolates have infected varying numbers of the other hosts.

Sansome (1938) has described how Reddick was able to improve the parasitism of *Phytophthora infestans*. He found "that the variety of potato President is resistant to *P. infestans*. But after two passages through President by artificial infection the degree of virulence of *P. infestans* is increased so that the lesions formed on President are as large as those formed on a susceptible variety, Green mountain. This higher virulence is kept up even after twenty passages through the susceptible variety." A modified method was adopted to improve the virulence of the isolates of *Colletotrichum*. They were grown on sterilised cotton seeds (strain H<sub>2</sub>) of *G. herbaceum* and after five passages through cotton seed, the cultures were used to inoculate cotton seedlings. The results were very interesting.

TABLE X

Statement showing the incidence of infection of cotton seedlings

Isolate	No. of seedlings inoculated	Total number infected on							
		3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
Cotton ..	16	4	16	—	—	—	—	—	—
Capiscum ..	16	—	7	11	13	14	15	—	—
Aristolochia ..	16	—	1	4	6	10	15	—	—
Curcuma ..	16	—	—	—	5	6	6	6	6
Cicer ..	16	—	7	10	14	15	—	—	—
Control ..	16	—	—	all healthy.					

The results indicate that all the isolates can be gradually 'educated' to become pathogenic on cotton seedlings which were not being infected originally, by growing the organisms on sterilised cotton seeds for a number of generations. All of them do not become equally virulent and there is a difference in the speed of infection (Plate III, Fig. 7).

Another experiment was conducted in which the cotton isolate was grown on sterilised cotton seed or *Capsicum* fruits for seven generations and then used for virulence tests on cotton seedlings. The following results were obtained.

TABLE XI

Statement showing the virulence of the cotton isolate after passage through cotton or *Capsicum*

Medium	No. of seedlings inoculated	No. of seedlings infected on									
		3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day
Cotton seed ..	20	12	20	—	—	—	—	—	—	—	—
<i>Capsicum</i> fruits	20	—	—	—	1	2	4	8	12	15	20
Control ..	20	—	—	—	all healthy						

The results show that the infective capacity of the cotton isolate becomes attenuated when grown on *Capsicum* fruits for a number of generations (Plate III, Fig. 8). When grown on agar media, however, the virulence is maintained for a much longer period.

#### DISCUSSION

It is evident from the studies described above that the taxonomy of the isolates of *Colletotrichum* under study at present classified as three or more different species, is in need of revision. It is seen that these isolates produce saltants very readily on agar media and such changes are bound to take place in nature also. The factors that have guided the erection of these species shall be reviewed and their validity examined.

The chief characteristics that are taken into consideration in defining species are the morphological characters, the dimension of the reproductive bodies, the acervuli and conidia and the pathogenicity of the isolate. These shall be examined one after another to assess the amount of reliability that can be placed on them.

The morphological characters of the isolates under study resemble one another very closely. If they were not properly labelled it will be difficult to distinguish one isolate from another. Ling and Lin (1944) state that "in comparison with a number of species of *Colletotrichum* such as *C. circinans*,

*C. indicum*, *C. truncatum* and *Glomerella glycines*, *C. capsici* differs from them in no essential way."

The dimensions of the acervulus fluctuate very much in the same isolate and consequently its size is not of much taxonomic value. Butler (1918) has recorded the size of the acervulus of *C. capsici* as 75–120  $\mu$ . The acervuli of the same species on the fruits of *Capsicum* collected locally have exhibited a fluctuation of 63 to 295  $\mu$  and on agar media the maximum reached was 315  $\mu$ . Ling and Lin (1944) state that the size of the acervulus of *C. capsici* on one host varied from 74–187  $\mu$  while on another host the variation was from 97–288  $\mu$ . A structure which exhibits such wide variation cannot be relied upon for specific differentiation.

The setae formed on the acervuli have been known to be definitely influenced by the environment and substratum to a large extent. Sometimes their formation itself is suppressed. Ikata (1937) and Ramakrishnan (1941) have indicated that the setae cannot be considered to be of any consequence for the purpose of specific differentiation. The shape and size of the conidium form important taxonomic characters. In the genus *Colletotrichum* the shape of the spore is useful in distinguishing certain species from others. The spores are either oblong, spindle-shaped, or falcate with tapering or blunt ends in different species, being more or less constant in the same species. The size is however influenced by the substrate and varies within limits. Yet its significance in specific differentiation cannot be ignored. Judged by these standards it is seen that all the isolates under study have similar mean dimensions of conidia and cannot be distinguished from each other either by the shape or size of the conidium.

An undue emphasis has been laid on the pathogenicity of the isolates of this genus in differentiating species. *C. capsici* was first recorded on *Capsicum*. Butler and Bisby (1931) have given a long list of plants serving as hosts for this species. They are: *Capsicum* spp., *Solanum nigrum*, *S. xanthocarpum*, *Datura fastuosa*, *Hibiscus esculentus*, *Cassia ensiformis*, fruit of *Vigna catjang*, *Dolichos lab lab*, *Solanum melongena*, *Citrus* sp. and *Carica papaya*. Ramakrishnan (1941) has observed the fungus on *Carthamus tinctorius*. Ling and Lin (1944) have noticed the fungus on fruits of *Lycopersicon esculentum* causing a fruit rot in China. A wide host range is thus established for this species. *C. curcuma* was described as causing leaf-spot of *Curcuma longa*, to which host it owes its specific name. Sundararaman (1925, 1926) carried out a number of cross-inoculations with this isolate and considered that the fungi on *Capsicum* and *Curcuma longa* belong to the same species,

Sundararaman (1922) has however erected a new species *C. zingiberi* (*Vermicularia zingiberi*) causing leaf-spot of *Zingiber officinale*. His decision was arrived at owing to (a) "the difference in the measurements of sporodochia between the *Colletotrichum* (*Vermicularia*) on ginger, turmeric, and chillies; (b) the character of the chlamydospores; and (c) the negative results in the cross-inoculations on chillies and turmeric." In the paper describing this species the measurements recorded of the acervuli (sporodochia) are 50 to 140  $\mu$  for *C. zingiberi* and 35 to 160  $\mu$  for *C. curcuma*. The former comes within the range of the latter and does not exhibit any difference. Appressoria (chlamydospores) are formed in all the isolates under study in the paper and no difference in their formation could be made out. It is questionable whether much importance can be attached to negative results of inoculation. In the absence of a thorough knowledge of the optimum environmental conditions necessary to produce successful infections there is every likelihood of failures of infection. The spore measurements were however found to agree with those of *C. capsici*.

Dastur (1934) has erected a provisional new species of *C. indicum* causing seedling blight of cotton. The only difference he found in this isolate when compared with *C. capsici* was in its pathogenicity. He found that the isolate from cotton did not infect *Capsicum* nor *C. capsici* cotton seedlings. But the infection experiments carried out at Coimbatore with the two fungi have shown that both the isolates can parasitize the two hosts.

It can be seen from the above that the occurrence of the fungi on different hosts and the variation in the pathogenicity of the isolates had prompted the creation of new species of *Colletotrichum*. Species of this genus are not obligate parasites but facultative saprophytes capable of leading a saprophytic existence in nature. Specific differentiation on differences of pathogenicity alone is not a reliable guide with such organisms. The substratum on which the fungus grows for a protracted period has been shown to influence the infective capacity of the isolates of this genus. Therefore the creation of new species on the variation of the pathogenic capabilities alone of the organisms cannot be approved. More reliance has to be placed on stable characters.

It is therefore concluded that all the isolates studied above should be included in one species. According to the rules of botanical nomenclature the name *C. capsici* has to be adopted being the earliest. *C. curcuma* and *C. indicum* have to be merged into this species. The reasons for creating the species of *C. zingiberi* (Sundararaman, 1922) are not tenable and this fungus has also to be brought under *C. capsici* which it resembles very much,

The author himself has stated that "there is a good deal of similarity among the ginger, chillies, and turmeric *Vermicularias* in point of spore measurements." These must be considered only as strains or races of *C. capsici*. This species has a wide host range, and it produces saltants freely and the different races met with in nature might have arisen in a similar manner. Being associated with a particular host for some period the infective capacity of the race on the particular host becomes pronounced. This accounts for the variability in the pathogenicity of the races.

I am grateful to K. M. Thomas, Esq., Government Mycologist, for all the help rendered in carrying out this investigation.

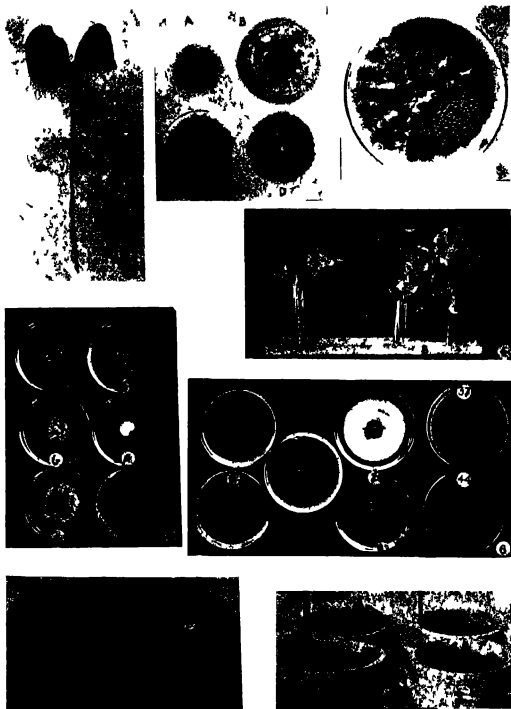
#### SUMMARY

Studies on the physiology of *C. indicum* Dast. were made. The fungus grows best in the neighbourhood of 32° C. The size of the conidium is not affected by temperature. Maltose and sucrose form the best sources of carbohydrates among those tried. Peptone serves as a good source of nitrogen. Staling products are formed in cultures over three weeks old. Filtrates of old cultures inhibit the growth of the fungus. Seedlings of cotton kept in these filtrates wilt in twelve hours.

A comparative study of *C. indicum*, *C. capsici*, *C. curcuma* and isolates from gram (*Cicer arietinum*) and *Aristolochia bracteata* was made. It was found that by growing the isolate on the tissues of a particular host for a number of generations its pathogenicity on that host is improved. Thus the various isolates under study were able to infect cotton seedlings when they were grown on sterilised cotton seeds for five generations. The taxonomic position of these isolates is discussed and it is concluded that all of them belong to one species, *C. capsici* Syd.

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# EXPLANATION OF PLATE

1. Cotton seedling affected by *Colletotrichum*.
2. Growth of *Colletotrichum* (Cotton strain) on
 

(a) Lactose.	(b) Maltose
(c) Glucose.	(d) Sucrose.
3. Salts of *Colletotrichum* (cotton) on Richards agar.
4. Effect of filtrate on cotton seedlings
 

(a) Filtrate	(b) Richards solution (control)
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5. Nitrogen sources and growth of *Colletotrichum* (cotton).
 

(a) Urea.	(b) Asparagin.	(c) Peptone.	(d) Potassium nitrate.
(e) Ammonium sulphate.	(f) Potassium nitrite.		
6. Effect of temperature on growth
 

10° C	32	37
5° C.	15° C.	20
		44° C
7. Cotton seedlings infected by (a) *C. capsici* (*Capsicum* strain), (b) *C. capsici* (*Aristolochia* strain) grown on cotton seeds
8. Cotton seedlings infected by *Colletotrichum* (cotton strain) (a) after 7 passages through *Capsicum*; (b) grown on cotton seeds

## ADDITIONS TO FUNGI OF MADRAS—I

By T. S. RAMAKRISHNAN AND K. RAMAKRISHNAN  
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Received October 10, 1946

(Communicated by Dr. B. V. Nath, C.J.E., D.Sc., F.R.I.C., F.A.Sc.)

DURING a foray undertaken in March 1946, collections of fungi were made in the jungles round about Coonoor and Ootacamund in the Nilgiris district of Madras province. Among the collections were several new records for the locality and some which are new to science. Three of these fungi are described below:

(1) *Xenostele neolitsea* sp. nov.

*Neolitsea zeylanica* Merr. is a medium-sized tree common in the upper slopes of the Nilgiris in the neighbourhood of Ootacamund, Kotagiri and Naduvattam. At the time of the visit this plant was affected by a severe epiphytotic of rust in all these places. Numerous brown rounded woody galls were seen on the leaves and sometimes on the petioles and stem (Plate, Fig. 1). The galls on the leaves are more conspicuous on the lower surface, there being few or none on the upper. They are isolated or clustered together and their diameter varies from 2 to 5 mm. But on the branches the hypertrophied portions are 2.5 cm. or more in thickness as against the normal thickness of half a centimeter of the healthy part. Wherever a gall develops on the lower surface of the leaf a corresponding depression is visible on the upper surface.

On the surface of the galls a number of rounded warts or tubercles are seen. The wart is ruptured and a whitish conical structure projects from the interior. In others a depression or cup-like cavity is present from which reddish brown mass of teliospores is visible. Two to thirty-two cups or more can be seen on old galls depending on their size.

The gall is very hard and woody. In cross-section the tissue of the gall is found to be made up of a high proportion of thick-walled scalariform cells mixed with parenchymatous cells packed with starch grains. Sunk in the galls are the cups of the teliosori. In the early stages a conical whitish to cream-coloured solid structure projects out of each sorus, bursting through the outer surface. When the galls are young a number of these whitish structures are seen protruding out. These can be easily picked out by needles and they come off as "stoppers". The conical structure represents the

peridium. When a section is examined the peridium appears as a solid mass made up of several layers of hyaline, thick-walled, sterile cells, rectangular to polygonal in shape and  $40-80 \times 17-19 \mu$  in size, closely united together and with a finely warty surface. In nature the peridial caps fall off after a time and expose the telia (Plate, Fig. B).

Telia are cup-shaped,  $300-400 \mu$  deep and  $300-410 \mu$  wide. A pseudo-parenchymatous mass of fungal tissue develops from the bottom of the cup. The upper layers of this tissue are made up of elongated cells closely packed together (Plate, Fig. C). The teliospores originate from these cells. The teliospores are stalked with long hyaline pedicels up to  $200 \mu$  in length. The pedicels easily break off leaving short remnants still attached to the spore. Each telium produces several spores borne in succession, the older ones being pushed up as new ones are formed. Thus an apparent resemblance to several layers of spores is produced. The remnants of the basal parts of the pedicels of old spores can be seen between the younger spores. Each teliospore is two-celled, elliptical or spindle-shaped, with rounded ends, dark reddish-brown and smooth-walled. The shape of the teliospore resembles a structure made of two cones united by their bases and having blunt apices. The spores measure  $47 \times 26 \mu$  ( $40-56 \times 24-29 \mu$ ). The upper cell varies in length from  $20-24 \mu$  and the lower from  $20-32 \mu$ . The cavity of the cells is bell-shaped. One germ pore is present in each cell—at the apex of the top cell and near the point of attachment of the stalk in the bottom cell. There is a slight constriction at the junction of the two cells, which in some cases becomes very pronounced. The two cells may separate from each other or be united only in the centre.

The telial stage alone has been observed in this rust. From its morphology it is manifest that it belongs to the genus *Xenostele* Syd. Two species of this genus have been recorded—*X. echinacea* (Berk) Syd. on *Actinodaphne molochina* in Ceylon and *X. Litsea* (Pat.) Syd. on *Litsea glauca*—in Japan. The galls formed by *X. echinacea* are developed only on leaves and the telial cups are  $200-250 \mu$  in diameter. Further the stalks of the spore are said to be golden-brown and twisted into bundles. The rust on *Neolitsea* produces galls on stems and leaves and the cups are bigger being  $300-410 \mu$  wide. The stalks are hyaline and not twisted into bundles. *X. Litsea* has been reported only on the leaves of *Litsea glauca* and the spores have a rough surface as described by Sydow (1920). The spores of the rust on *Neolitsea* are quite smooth and no warts have been seen even when they are examined with an oil-immersion objective. Since the host is different and there are differences in the size of the sorus, the nature of the pedicel and the wall of

the spore this rust is considered to be different from the other two species and is named *X. neolitsea*

*Xenostele neolitsea* sp. nov.—Aecia, pycnia, and uredia not known; telia sunk in woody galls formed on leaves and stem, 300–410  $\mu$ , with a whitish conical peridium of many layers of sterile, rectangular to polygonal, thick-walled, and warty cells, teliospores two-celled, dark reddish-brown spindle-shaped, sometimes separating into individual cells, 47–0  $\times$  26 0  $\mu$ ; pedicelled, pedicels long up to 200  $\mu$ , hyaline fragile

*Habitat*—On living leaves of *Neolitsea zeylanica* Merr on the Nilgiris, March 1946 Collected by C L Subramanian and K Ramakrishnan (type) at Ootacamund, 15th March 1946 Type deposited in the herbarium of the Government Mycologist Coimbatore and Herb Crypt Ind Orient New Delhi

Aecia, pycnia et uredia non cognita, telia demersa ligneis excrescentibus formati in foliis et caulibus 300–410  $\mu$ , albo conico peridio multorum stratorum cellarum sterilium rectangularium ad polygonarum crassomuratum, echinularum, telio-sporidia bicellata fusci rubricosi brunnei coloris, fusiformia, interdum separantia in duas cellas, 47  $\times$  26  $\mu$ , Pedicellata, pedicelli longi hyalini fragiles, ad 200  $\mu$

Hab in vivis folii et rames *Neolitsea zeylanica* Merr Ootacamund (Nilgiris) 15-III-1946 C L Subramanian et K Ramakrishnan typus Typi specimina deposita in Herbario Government Mycologist Coimbatore et Herb Crypt Ind Orient, New Delhi



FIG 1 Teliospores  $\times$  720

(2) *Pseudopeziza rubra* sp. nov

*Rubia cordifolia* L. a common climber was affected by a leaf spot in the neighbourhood of Lovedale and Coonoor (Nilgiris District of Madras

Province) On the under surface of the spotted region groups of apothecia had developed. Four to five apothecia were present in each group. These apothecia were saucer-shaped 0.5–0.8 mm in diameter, roughly circular with incurved margins. When fresh the texture of the apothecium is waxy but it becomes hard and brittle on drying. Young apothecia have a light buff colour but older ones turn dark on the upper surface. The apothecium opens out when mature.

A section of the leaf through the apothecium (Plate Fig. D) reveals that the latter is sub-epidermal in origin though the whole apothecium is outside the leaf and is carried on a short stalk-like structure  $83\mu$  in length and  $125\mu$  in breadth. This portion broadens out into the hypothecium composed of fairly large polygonal thin-walled cells. Above the hypothecium and below the hymenium is the narrow subhymenial layer formed of small-celled tissue. The hymenium is made up of closely packed asci and paraphyses. The asci are hyaline more or less clavate and  $70 \times 5.2\mu$  ( $59-93 \times 4-7\mu$ ). The ascospores are eight in number, uniseriate and obliquely arranged. They are hyaline long-oval and  $7 \times 2.5\mu$  ( $5.5-9 \times 1.5-5\mu$ ). The paraphyses are as long as the asci, filiform, unbranched at the tip and hyaline.

*P. repanda* (Fr.) Karst. has been recorded on the leaves of *Galium* spp. (*G. mollugin*, *G. boreale*, *G. silvaticum* and *G. asperula*) (Butler and Bisby 1931 and Saccardo 1889) and leaves and stem of *Rubia tinctorum* (Saccardo 1889 and 1898) belonging to the Rubiaceae and *Potentilla cerasti* belonging to the Rosaceae. On the last host the apothecia are seen on the stems and rarely on the leaves. But the species now recorded is different from *P. repanda*. It has bigger apothecia and larger asci. Further the size, shape and arrangement of the ascospores are entirely different from those of *P. repanda*. Therefore it is named *Pseudopeziza rubia*.

*Pseudopeziza rubia* sp. nov. Apothecia hypophyllous, gregarious, light buff when young and dark when old, concave, roughly circular 0.5–0.8 mm in diameter, asci, hyaline, long-clavate  $70.0 \times 5.2$  ( $59-93 \times 4-7$ )  $\mu$ , ascospores uniseriate, hyaline, 8, oblong— $7 \times 2.5\mu$  ( $5.5 \times 1.5-5\mu$ ), Paraphyses filiform, unbranched, hyaline.

**Habitat**—On living leaves of *Rubia cordifolia* L. Lovedale and Coonoor 19-3-46. Collected by C. L. Subramanian and K. Ramakrishnan. Type deposited in the herbarium of the Government Mycologist, Coimbatore and Herb. Crypt. Ind. Orient., New Delhi.

Apothecia hypophylla, gregaria, leviter brunnei coloris in juventute, testaceo cooris in maturitate, concava, circiter orbicularia 0.5–0.8 mm in

diametro, asci hyalini, elongato clavata  $70.0 \times 5.2$  ( $59-93 \times 4-7$ )  $\mu$ ,  
 asco sporidia uniseriata hyalina 8 oblonga  $7 \times 2.5$  ( $5.9 \times 1.5-5$ )  $\mu$ , para-  
 physes filiformes simplices hyalini

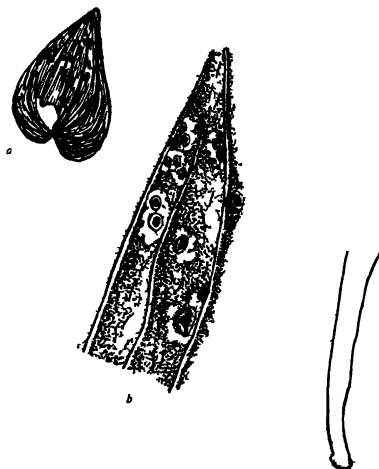


FIG 2 a leaf with apothecia b a portion enlarged c paraphyses and ascus  $\times 2000$ .

*Habitat* in vives folius Rubiae cordifoliae L Lovedale, Coonoor  
 19-3-46. Log. K. Ramakrishnan et C L Subramanian Typi specimen

deposita in Herbario Government Mycologist Coimbatore et Herb Crypt Ind Orient New Delhi

(3) *Puccinia Linkii* Koltzch

This rust was recorded on the leaves of *Viburnum erubescens* Wall

Telia are epiphyllous and brown spots are visible on the lower surface of the leaves. Very often telia are ring shaped erumpent and dark brown in colour. Teliospores are pedicellate pedicels hyaline. They are two celled chestnut brown with hyaline prominent sparsely arranged warts on the wall. They measure  $42 \times 17.5$  ( $30.75$   $51 \times 10.2$   $26.7$ )  $\mu$ . They are elliptical with rounded ends slightly constricted in the middle with one germ pore in each cell—at the apex of the top cell and at the junction of the stalk in the lower cell.

*Puccinia Linkii* Koltzch has been described (Sydow 1904) on *Viburnum pauciflorum* in America. The rust on *V. erubescens* resembles that closely and is therefore identified as *P. Linkii*.

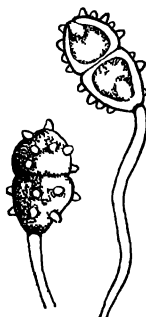


FIG. 3. Teliospores ( $\times 720$ )

The authors acknowledge the help rendered by Mr. M. S. Balakrishnan, Research Fellow, Mycology Section, in making drawings and by Rev. Fr. Singarayar of St. Joseph's Seminary, Coimbatore, in rendering the diagnosis into Latin. Dr. B. B. Mundkur of New Delhi and Mr. K. M. Thomas

Government Mycologist, Coimbatore, were kind enough to go through the manuscript and we are grateful to them for their help

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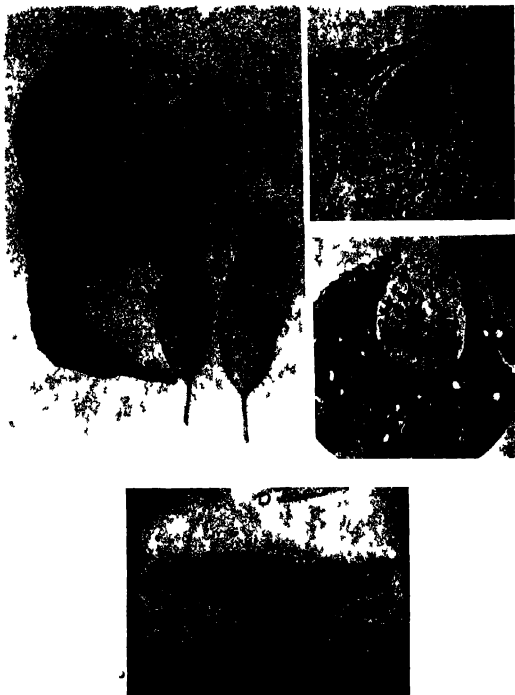
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## EXPLANATION OF PLATE

- A *Neolusea zeilanica* leaf and stem showing the galls produced by the rust (Natural size)  
 B Section through the gall showing the telial cup closed by the stopper like peridium ( $\times 100$ )  
 C A similar section showing the telio-sorus and the teliospores ( $\times 100$ )  
 D Section of apothecium of *Pseudopeziza rubra*

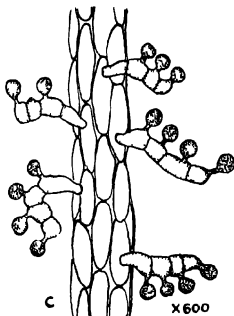
*I S Ramakrishna  
and K Ralakrishnan*

*Pro Ind Acad Sci B 1 XXV Pl IV*





A

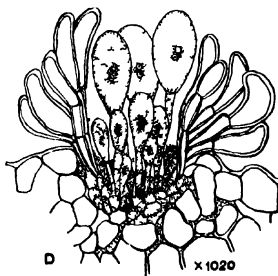


C

X 600



B



D

X 1020

## A NEW RUST ON *PREMNA TOMENTOSA* WILLD.

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Received September 11, 1946

(Communicated by Rao Bahadur Dr. B. Viswanath, D.Sc., F.R.I.C., C.L.E., F.A.S.S.)

*Premna tomentosa* is common in the foot-hills of the Nilgiri mountains, in the neighbourhood of Kallar (Coimbatore District). In the months of December and January it is affected by a rust.

The uredosori are formed on the lower surface of the leaves. They are minute, crowded, pulverulent, mixed with the tomentum of branched hairs and in mass having a tawny olive colour. The sorus bursts through the epidermis. A peridium is lacking but two or more rows of paraphyses are present all round the periphery of the sorus. These are incurved forming a pseudoperidial structure (Figs. A and D). The paraphyses are one to two septate, with the terminal cell long, often bent and club-shaped. The wall is irregularly thickened and light yellowish brown, rarely hyaline. The uredospores are borne singly on stalks. They are oval or elliptical, prominently echinulate, brownish yellow in colour but with a hyaline spore wall. The spores measure  $29.5 \times 19.5 \mu$  (the range being  $18.6 - 31.0 \times 15.5 - 21.7$ ).

The teleuto-sori are hypophyllous and columnar. The telial columns are solitary but produced near each other. They originate from the sub-epidermal portion and are surrounded by two or more rows of clavate, incurved brown paraphyses as in the case of uredosori. The columns are filiform, tendril-like and many of them are intertwined. Each column is about 5-6 mm. in length and  $25-35 \mu$  in thickness made up of 5-7 rows of closely united cells. The teleutospores are one-celled, sessile, oblong, yellow-ochre in colour and measure  $28.5 \times 8.7 \mu$  (range being  $17.1-44.9 \times 4.7-12.4$ ). All the spores are closely united together (Plate V, Fig. B).

The teleutospore is capable of immediate germination. When portions of the telial columns are floated on drops of water kept on a slide inside a moist chamber, germination takes place in 8-10 hours at room temperature ( $28^{\circ}\text{C}$ ). A stout, 4-celled basidium grows out of the spore. From each

cell a short sterigma develops and on this a hyaline round or oval basidiospore is formed (Plate V, Fig. C).

Petch (1911) has described *Cronartium premnae* on *Premna corymbosa* R. and Willd from Ceylon. Sydow (1918) amended this as *Crossopsora premnae* (Petch) Syd. The uredospores of this fungus are stated to be 20-28 by 16-19  $\mu$ ; thick-walled, hyaline clavate paraphyses are present in the uredosorus. The teleutosori are several millimetres in length and about 50  $\mu$  in diameter and the spores are 40-58  $\times$  8  $\mu$ .

The rust on *Premna tomentosa*, however, belongs to the genus *Crossopsora*. Since the uredosorus has no peridium but has a ring of incurved paraphyses round it, this rust cannot be a *Cronartium* but only *Crossopsora*.

The rust on *Premna tomentosa* differs from that recorded on *P. corymbosa* in having thinner telial columns and smaller teleutospores. Since it has not been recorded before and is new it is described as *Crossopsora premna-tomentosa*.

*Crossopsora premna-tomentosa* sp. nov.—Uredosorus hypophyllous, minute, crowded, erumpent, pulverulent, with a ring of incurved, 1-2 septate light-brown paraphyses; uredospores oval to elliptic, echinulate, contents brownish yellow, wall hyaline, 29.5  $\times$  19  $\mu$ ; teleutosorus hypophyllous, filiform, surrounded by a ring of several rows of paraphyses at the base, dark brown, 5-6 mm. in length, 25-35  $\mu$  in diameter, teleutospores sessile, one-celled, united, oblong, yellow-ochre in colour 28.5  $\times$  8.7  $\mu$ .

*Habitat*.—In living leaves of *Premna tomentosa* Willd. at Kallar, Coimbatore District, January 6th, 1946 (Soumini and Krishnamurthy). Type specimen deposited in the herbarium of the Government Mycologist, Coimbatore.

*Crossopsora premna-tomentosa*.—Uredosoris hypophyllis, minutis, gregariis, erumpens; pulverulentus, paraphysibus, numerosus, 1-2 septatis, introrsum curvatus, levi brunneis; uredosporis, ovatis, v. ellipsoideis, echinulatis, flavo brunneis, 29.5  $\times$  19  $\mu$ ; episporio hyalinis; teleutosis, hypophyllis, filiformitibus, circumdatum annulo gradum multorum paraphysium basi, fuscum, 5-6 mm. long, 25-35  $\mu$  lat.; teleutosporis arcticonnexis, oblongatis, 28.5  $\times$  8.7  $\mu$ , flavus ochraceus.

*Hab.* in vivis foliis *Premna tomentosa* Willd. Kallar, Coimbatore District, 6-1-1946 (Soumini and Krishnamurthy).

We are indebted to Mr. C. S. Krishnamurthy for helping in the collection of specimens, to Mr. S. N. Chandrasekhara Ayyar for the identification of the host and to Rev. Fr. Singarayar of St. Joseph's Seminary, Coimbatore, for the Latin translation of the diagnosis.

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EXPLANATION OF PLATE

- A. Photomicrograph of a uredosorus ( $\times 400$ ).  
B. Photomicrograph showing portions of telial columns and some uredospores ( $\times 400$ ).  
C. Germination of teleutospores ( $\times 600$ )  
D. Drawing of a section through a uredosorus ( $\times 1020$ )



## FRUIT ROT OF TOMATOES CAUSED BY *PHYTOPHTHORA PALMIVORA* BUTL.

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Received June 15 1946

(Communicated by Dr B Viswanath CIE DSC FRIC)

DURING the north-east monsoon period in 1944 and 1945 a fruit rot disease of tomatoes was in evidence at the vegetable production centre at Tidiyalur, Coimbatore district. The crop was raised in a field where the plants were not propped. The disease became evident soon after heavy rains in October. Since the propping was not done several branches were spreading on the ground and consequently some of the fruits borne on these branches were at times in contact with the wet soil. Such fruits were the first to be affected. Nearly 25 per cent of the fruits were involved. The disease later on spread to fruits borne on higher branches also.

The disease was observed mainly on the fruits. In a few instances the young shoots touching the soil were also affected. The stem and the branches at this region were first discoloured with a dull green water soaked appearance but later these turned dark brown and rotted. Fruits of all sizes were affected. On green fruits the disease commences at the blossom end or at the side which touches the soil in the form of small water-soaked spots. These increase rapidly in size and in the course of 3 to 4 days the entire fruit becomes involved. The fruit assumes a brownish colour, is soft to the touch and the skin easily peels off. In wet weather the fungus grows out and forms a whitish fluffy growth on the surface. Sometimes, concentric markings may be seen in the affected portions and the external fungal growth also assumes similar distribution (see photograph). The organism causing the disease was found to be a *Phytophthora* and numerous sporangia were detected in scrapings of the external growth.

Fruit rots of tomato caused by *Phytophthora* have been recorded from all over the world. Tucker (1933) has recorded a rot of fruits near or in contact with soil as the most common type of infection caused by *Phytophthora parasitica*. Dast Reddick (1920) has described the occurrence of a disease in glass houses in New York causing girdling of the stem and rapid rotting of fruits due to *P. parasitica*. Dast. *P. infestans* de Bary has been known to infect fruits in various countries (Tucker, 1933). Buckeye rot of

tomatoes in California is ascribed to *P. dreschleri* Tucker and *P. capsici* Leonian (Tompkins and Tucker, 1941). Lavellée (1941) has recorded *P. parasitica* Dast as responsible for buckeye rot. Thus tomato fruit rot is widespread and is reported to be caused by different species of *Phytophthora*.

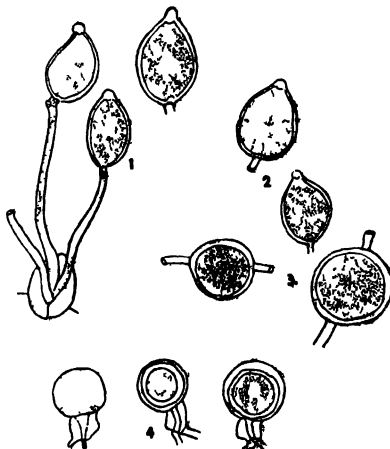


FIG. 1. Sporangia from tomato fruits ( $\times 400$ ).

FIG. 2. Sporangia from culture ( $\times 400$ ).

FIG. 3. Chlamydospores from culture ( $\times 400$ ).

FIG. 4. Zygospores formed in combination with another strain ( $\times 400$ ).

The organism causing the disease at Coimbatore was isolated into pure culture from a single sporangium. It grew well on oat and french-bean agar media. Numerous characteristic sporangia and chlamydospores were produced. The sporangia are oval or pear-shaped, papillate, mainly terminal and measure  $33.4 \times 22.6 \mu$  ( $18.6-46.5 \times 15.5-37.2 \mu$ ) (Figs 1 and 2). The chlamydospores are spherical, hyaline or light yellowish brown in

colour. The colour is developed in the wall of the older chlamydospores. They were formed terminally or more often intercalary and measured  $23.4 \times 20.5 \mu$  ( $15.5-31 \mu$ ) (Fig. 3). Oospores were not formed.

The pathogenicity of the organism was established by inoculation experiments of the fruits on growing plants and detached fruits kept in sterilized moist chambers. The plants with the fruits (grown in pots) were placed in glazed cages kept moist by having a layer of moist sand at the bottom and frequent sprayings with sterile distilled water. The fruits were young and green. All the inoculated fruits were infected and in 5 days they rotted completely. The detached fruits were green, bigger and more mature. These were infected in 3 days and completely rotted in 6 days. In both cases the same fungus was recovered from the infected fruits. The controls remained healthy throughout.

The organism is able to infect young branches and leaves of tomato. These become involved in a blackish green wet rot and the rotten portions fall off or the stem breaks at the point of infection. With the severance of the infected branch or stem the spread of infection is arrested.

The organism infects the fruits through unwounded surfaces. Inoculation experiments showed that infection can take place through any part of the fruit. Bits of culture were placed on the fruit near the stalk, styler end and other portions of the fruit and in all cases infection occurred. The hyphae ramify through the tissues of the fruit being both inter-cellular and intra-cellular. The affected tissues became soft and discoloured.

The average dimensions of the sporangia and chlamydospores of this *Phytophthora* agree with those of *P. arecae* (Coleman), Pethybridge, *P. palmivora* Butler, *P. meadu* McRae and *P. parasitica* Dastur Tucker (1931) is of opinion that "the dimensions of sporangia considered independently of other characters cannot be accorded much importance taxonomically". The same may be said to apply to the dimensions of chlamydospores also.

The fungus was grown in paired culture with two strains of *Phytophthora* isolated from arecanut and kindly supplied by Dr. Uppal, Plant Pathologist, Bombay. Oospores were produced with one of these strains but not with the other. The oospores were spherical, yellowish in colour and measured  $20.0 \mu$  in diameter (range  $15.5-24.8 \mu$ ) (Fig. 4).

The isolate from tomato closely resembles one of the strains isolated by Uppal and Desai (1939) from arecanuts (Nilekan strain) and is similar to the strain found on arecanut in South Kanara. A more detailed study including all the South Indian isolates of *Phytophthora* and others obtained

from elsewhere is being carried out in this laboratory and will form the subject of a further communication.

#### CONTROL

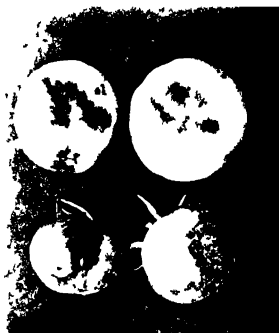
Experience in other parts of the world has shown that when the tomato plants are sprayed with Bordeaux mixture the fruit rot disease is controlled. Further it is noticed that the disease commences on the fruits lying in contact with the soil. If it is possible to prevent this, the incidence of the disease can be lowered. This can be accomplished by staking the plants or tying them to frames and thus prevent them from trailing on the ground. Further the plants and fruits must be sprayed with Bordeaux mixture.

#### SUMMARY

A fruit rot of tomatoes was prevalent in Coimbatore during the rainy season. Fruits in contact with the soil were the first to be affected. *Phytophthora* was isolated from these fruits. The fungus was found to resemble *P. palmivora* Butl. (areca strain from S. Kanara).

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Development of fruit



## **CALIGUS SCIAENAE N. SP. PARASITIC ON SCIAENA GLAUCA FROM MADRAS**

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Received January 13, 1947

(Communicated by Prof S G M Ramanujam, F A S c)

As against the exhaustive studies of British parasitic copepods by Scott,<sup>7</sup> of American forms by Wilson<sup>8</sup> and European species by Brian<sup>9</sup> and Hansen,<sup>4</sup> our knowledge of copepods parasitic on Indian fishes is poor. Bassett Smith,<sup>1</sup> Kirtisinghe,<sup>5</sup> Thompson,<sup>6</sup> Wilson<sup>8</sup> and Brian<sup>9</sup> and Grey have recorded and described a few forms. Hence a full description of this parasitic caligid copepod was deemed not superfluous.

This parasite was found attached to the tip of a gill filament of the first gill of *Sciaena glauca*. It measures 1.7 mm, the setae of the anal plates included. (The frontal area is 1 mm, the cephalothorax .7 mm; abdomen .7 mm; the anal plates and setae .2 mm.) The frontal region is marked by the possession of two large lunules visible even dorsally. Examined ventrally each sucker appears like a deep spherical cup. The rim of the cup is turned in to form a flat shelf. This edge of the sucker is not entire, being cut up anteriorly and the two cut ends overlapping each other to a slight extent. The entire lunule is clearly formed by the folding of the edge of the frontal plate. Medially the anterior border of the frontal area shows a projection on the ventral side. This projection is a median sucker-like fold of the frontal edge and occurs just where the frontal filament would have been during the Chalimus period in the development. The persistence of this sucker along with the well-developed lunule shows that it has just passed the Chalimus stage. The frontal area is also marked by the occurrence of the 1st antennae whose basal joint appears continuous with it.

The cephalothorax is almost circular in shape being .7 mm long and .75 mm broad (Fig. 1). The cephalic area is marked off from the thoracic area by a semi-circular groove. The carapace as well as the rest of the body appears whitish, transparent and free from colour marks. The posterior edge of the cephalothorax area extends dorsally over the free thoracic segment which is clearly visible from the ventral aspect. With the convex form of the cephalothoracic shield and the flattened or slightly hollowed form of the sternal plate of the third thoracic segment, a cupping adhesion

can be effected by the body of the parasite whenever necessary. The fact that the cephalothorax is formed of ten segments (the seven-segmented cephalon as well as three segments of the thorax united with it) is obvious from the ten pairs of appendages found on the ventral side. On the dorsal side the double median eye can be made out.

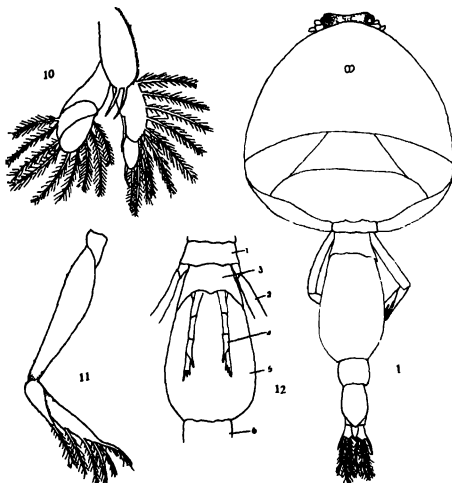


FIG. 1. Dorsal view of *Caligus scense*  $\times 73.3$ .

FIG. 10. III Swimming leg.

FIG. 11. IV Swimming leg.

FIG. 12. V Thoracic legs and the genital segment:

- |                           |                              |
|---------------------------|------------------------------|
| 1 Free thoracic segment.  | 4. Ventral fifth leg         |
| 2. Fourth swimming leg.   | 5 Genital segment.           |
| 3 Fifth thoracic segment. | 6. Second abdominal segment. |

**1st Antenna** (Fig. 2) is three-jointed, the basal joint being as broad as the lunule itself. It is heavily armed with about fifteen stout spines while

**Caligus sciaenae N. Sp. Parasite on Sciaena glauca from Madras 45**

the distal joint is long, slender and bears two spines on its body and four spines at its distal end

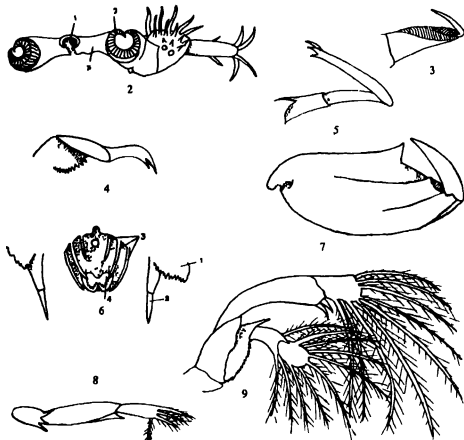


FIG 2 Frontal Plate and Antenna 1 median sucker 2 lunule 3 frontal plate

FIG 3 II Antenna

FIG 4 I maxilla

FIG 5 I Maxillipede

FIG 6 II Maxillae and mouth tube

1 Exopod of maxilla 3 Chitinous rods of mouth tube

2 Endopod of maxilla 4 Mandible

FIG 7 II Maxillipede

FIG 8 I Swimming leg

FIG 9 II Swimming leg

**2nd Antenna** (Fig 3) is two-jointed, the proximal joint being stouter than the distal, which is long, slender and terminates in a sharp curved spine. On the inner side are seen two grooved plates.

*Mandible* is long and slender, toothed like a saw with the distal tip curved inwards. The mandibles are connected with the mouth tube, in the posterior part of which the teeth at the tips of the mandibles can be made out. The lateral walls of the tube are supported by three rods on each side, there being no transverse rods supporting the lower lip. The mouth is reniform in outline.

*1st Maxilla* (Fig. 4) is attached more outwards than the base of the second antenna but a little posterior to the front edge of the mouth tube containing the mandibles. It consists of two distinct joints. The basal joint is short and stout made stouter by the occurrence of toothed plate or lamina representing the exopodite or palp (F. Scott). The distal joint is very decidedly hooked and with its two sharp recurved spines must help in attachment. Wilson described only a simple claw; the occurrence of two in the parasite is noteworthy.

*2nd Maxilla* which is nearly as long as the first maxilla has a columnar base and tapers to a blunt point (Fig. 6). A small many-toothed lamina at the base probably represents the exopodite of this appendage (Wilson describes two setae and considers them as endopodites and the main structure as the exopod).

*1st Maxillipede* (Fig. 5) is a very prominent appendage. It is distinctly three-jointed. The first two joints are long and stout while the third is longer and more slender, and appears to be capable of considerable movement. It ends in three sharp claw-like spines. There is a single-toothed plate at the base, indicating the endopod.

*2nd Maxillipede* (Fig. 7) arises nearer the mid-line and consists of two distinct joints. The basal joint is large and swollen and flattened. It bears a distinct tooth on its anterior border in this form, the terminal claw-like part folding like a knife-blade. At the outer part of the stout basal joint where the rest of the limb folds back can be seen a toothed bony plate rising from the posterior border of the base. At the very bottom of the basal joint on the medial aspect can be seen another toothed plate, not unlike those described before in the maxillae. As this occurs on the medial aspect it is probably homologous to the endopod.

*1st swimming leg* (Fig. 8) as well as the second and third swimming legs indicate the thoracic segments which have united with the cephalon. The first leg however is uniramous like the fourth leg. It is three-jointed. The basal joint is short and stout. Its outer margin bears three sharp spines while the fourth spine borne by the body projects tailward. The second joint is longer by half the length of the first and bears a spine at its outer

*Caligus sciaenae* N. Sp. Parasitic on *Sciaena glauca* from Madras 47

margin. The distal joint bears three straight spines pointing outwards and a fourth spine directed backward. These spines are provided with fine hairs. The second and third joints represent the exopod.

*2nd swimming leg* (Fig. 9) has a short stout basal joint bearing both the exopod and the endopod. The inner ramus of the limb is three-jointed. The first joint has a toothed posterior edge and bears a spine at its outer margin, the second joint is shorter and bears a tooth at its outer edge while the third is flat, broad and orbicular. It is fringed with nearly ten long plumose setae. The exopod is longer chiefly due to the first two joints which are similar to those of the endopod. The third joint bears on its posterior edge about the middle of its length, two short spines and at its distal margin bears eight long plumose setae.

*3rd swimming leg* (Fig. 10) is attached farther from the mid line because of the enlargement of the sternal plate of the segment. The natatory function of this appendage is unmistakable for even the basal segment is broad and flat. It is fringed on the outside by numerous short hairs and also bears two long plumose setae on the ventral side of the outer edge. The endopod is foliaceous and three-jointed. The outer two joints bear nearly ten long plumose setae. The exopod is also three-jointed, the outer two joints bear nearly ten long plumose setae.

*4th swimming leg* (Fig. 11) is uniramous, the endopod being absent. It is four-jointed. The basal segment is stout as in the other legs. The first joint of the exopod is by far the longest forming nearly half the length of the limb. The second joint is short but is produced into a long spine at its outer margins while the third is slender bearing four apically directed spines with a fifth spine at the distal end. All the spines or setae are plumose.

*5th swimming leg* (Fig. 12) represents a fifth thoracic segment as pointed out by Wilson. This occurs on the ventral side of the "genital segment". But as can be seen in the form described in this paper, these appendages really spring from the front part of the "genital segment". This anterior part of the segment is separated from the genital segment proper by a distinct groove. Therefore the genital segment is regarded as really the 1st abdominal and not as the fifth thoracic segment as Wilson has done.

*The abdomen* is three-jointed. The genital segment is nearly twice as broad as the succeeding segment, and nearly four times as long. There are no vestiges of appendages or other indications to show that this genital segment may be a composite of two segments fused into one. The segment behind it is broader than long while the third and last segment is longer than

broad. It ends in an obtuse point on either side of which the anal plates occur. Each lamina bears three long plumose setæ on its posterior side and two stouter spines one at each of the posterior corners. The absence of special structures on the antenna and of special plates on the 1st maxilliped usually used for prehension by the male make it probable that the parasite is a female. The size of the genital segment also does not contradict such a conclusion. The persistence of the median sucker (the relic of the frontal filament of the *Chalimus* stage in development) as well as the absence of any trace of the egg strings both argue the immature condition of the subject. This also serves to explain the occurrence of the fifth pair of thoracic legs (though in an unusual condition and position being pressed against the body) whereas these usually disappear in a mature female. The occurrence of this species of *Caligus* on *Sciana glauca* of the Madras Coast is noteworthy since *Caligus* (*Scianophilus* Van Beneden) *Benedeni* sp. nov. described by Bassett Smith<sup>1</sup> was taken from *Sciana diacanthus* from Bombay and later recorded from Ceylon by Thompson<sup>7</sup>. This species *C. Benedeni* differs from the form described in this paper in having the cephalothorax only a fifth of the whole length and being much less broad than the genital segment, the lunules being very small, the basal part of the 1st antenna having only twelve plumose setæ and the second joint having two long spines, the 1st pereopod having three long end bristles and three moderately long plumose setæ on its posterior border, the genital segment being rather long than broad, and the abdomen being single jointed. In view of these differences the present form is described as *Caligus sciænæ* n. sp. in this paper.

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**(I) THE INTERACTION BETWEEN IONS DRUGS  
AND ELECTRICAL STIMULATION AS INDICATED  
BY THE CONTRACTION OF AVIAN UNSTRIATED  
MUSCLE. (II) ACTIVE ELONGATION OF  
UNSTRIATED MUSCLE**

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AND  
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Received December 11, 1946

THE experiment on *Mytilus*, frog and mammalian muscles have been continued on the unstriated muscle from the domestic fowl to see if any differences exist. The body temperature of the birds is slightly higher than that of mammals and this might result in certain reactions. In mammals the differences between the responses of their unstriated muscle from frog unstriated muscle may be ascribed to: (1) greater ionic content of the saline, (2) to greater body temperature. The latter results in slower adaptation which increases sensitivity, while the former produces opposite results.

**EXPERIMENTAL**

The saline used was as previously (Singh, 1939). The experiments were performed at room temperature (25-30° C) as this range is optimum for most reactions. The muscles used were the duodenum and the œsophagus of the domestic fowl. The duodenum forms a U-loop enclosing the pancreas, blood vessels and nerves. One limb of the U-loop may be taken out with or without the attached pancreas and nerves; it provides a straight portion of the gut. The œsophagus was chosen, as its responses were exactly like those of the other unstriated muscle; a muscle nerve preparation was made.

**RESULTS**

The reactions of avian unstriated muscle resemble those of the other unstriated muscle, with the exception of a few differences.

*Effect of temperature.*—The optimum temperature for alternating current for the duodenum is 29-30° C. and for the œsophagus, 25-26° C. This lower temperature for the œsophagus is presumably due to its exposed position (Singh, Singh and Muthana, 1946). In the dog stomach, the optimum temperature at Bombay, which has similar climate as Karachi, was 24-25° C.

The higher optimum temperature in the fowl is presumably related to its higher body temperature. The optimum temperature for potassium is 20° C and for acetylcholine is 25° C, in the dog the optimum temperature for acetylcholine being 30° C. The optimum temperature is however variable (Singh and Rao, 1940). This appears to be due to adaptation, Fig. 1 (Singh, 1946). Tone may increase or decrease at high temperatures

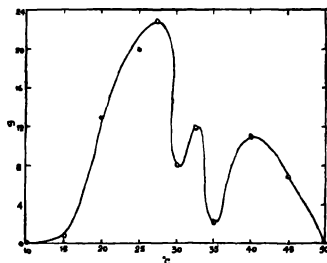


FIG. 1. *Fowl Duodenum*—The effect of temperature on A.C. contraction

(35–39° C). The increase resembles as in other unstriated muscle, and the decrease is probably due to adaptation, which increases at higher temperatures (Singh 1940). The duodenum is more affected by cold than other unstriated muscles, a few degrees increase in temperature above 25° C may produce great increase in excitability to alternating current.

**Effect of osmotic pressure**—The increase in osmotic pressure of the saline by addition of sucrose or sodium chloride beyond 20 to 40% above normal is depressant. The results of increase up to 20% are variable. In the *α*-ophagus increase in osmotic pressure by addition of sucrose to the saline increases the response to alternating current, decreases tone and the response to acetylcholine and potassium. With sodium chloride the response to alternating current decreases and that to potassium and acetylcholine is variable. In the duodenum, with sucrose, the response to alternating current increases, tone decreases, the response to acetylcholine increases and to potassium is variable. These effects are similar to those in *Mytilus* muscle.

Decrease in osmotic pressure of the saline by 20% in the œsophagus increases the response to alternating current, decreases tone and the response to potassium and acetylcholine, in the duodenum the response to alternating and tone is increased, and to potassium and acetylcholine is decreased. Further reduction in osmotic pressure is depressant to all in the œsophagus as well as duodenum, though tone in the latter may increase.

*Effect of calcium*—In the œsophagus the optimum concentration of calcium for alternating current, acetylcholine, potassium, and nervous stimulation is  $0.00206\text{ }M\text{ }CaCl_2$ , though the œsophagus may become hyper-irritable in the absence of calcium. In the duodenum the optimum concentration for alternating current is three to four times that in the œsophagus but for potassium and acetylcholine it is the same. As in the dog stomach excess of calcium up to  $0.02\text{ }M\text{ }CaCl_2$ , both in the duodenum as well as œsophagus may potentiate the response to acetylcholine and potassium.

Strontium acts like calcium, barium produces tonic contraction and so causes depression of excitability. Magnesium is depressant.

*Effect of lithium*—Replacement of sodium of the saline with lithium produces effects of sodium deficiency. Replacement of 20–40% of the sodium increases the response to alternating current, potassium and acetylcholine, tone decreases.

*Effect of sodium*—Replacement of part of the sodium chloride (20–40%) increase the response to alternating current, potassium and acetylcholine. Further increase is depressant. Complete removal of sodium chloride causes contraction, so also isotonic sucrose, suggesting that difference in ionic concentration on two sides of the membrane causes contraction.

*Effect of ammonium*—The replacement of the 20% of sodium of the saline with ammonium decreases the response to alternating current but increases that to potassium and acetylcholine. Further increase is depressant. Ammonium thus potentiates the response to potassium and acetylcholine, and may cause contraction. Withdrawal of ammonium may cause contraction.

*Effect of potassium*—The optimum concentration of potassium for the response to alternating current, potassium and acetylcholine is  $0.0016\text{ }M\text{ }KCl$ . Further increase is depressant and causes tonic contraction. The gut muscle is rather sensitive to potassium.

*Effect of hydrogen ions*—The optimum pH for alternating current in the duodenum is 8; at pH 6 it may become inexcitable. In the œsophagus this is also the optimum, but it may cause depression owing to increase of

tone, then the excitability declines as the pH is decreased from 9.24 to 8 and then increases up to pH 7 (Fig 2) Tone decreases with increase in hydrogen ion concentration. Increase in hydrogen ions do not potentiate the response to potassium and acetylcholine.

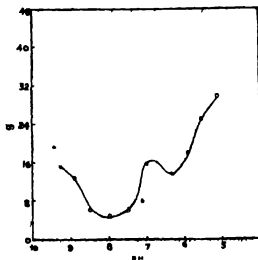


FIG. 2. *Fowl Oesophagus*.—The effect of pH on A. C. contraction

*Effect of anions.*—Small concentrations of Br,  $\text{NO}_3$ , I, SCN increase and large concentrations decrease the excitability, when they cause tonic contraction.

*Effect of eserine.*—There is a marked difference between the reactions of the fowl gut and oesophagus on one hand and the dog stomach on the other hand. It does not cause marked increase in excitability in the former as it does in the latter; it rather causes depression. It does not potentiate the response to acetylcholine; if at all, the potentiation is insignificant (*cf.* Brown and Harvey, 1938). It may cause depression. 1 in  $10^5$  potentiates the response to alternating current.

*Effect of adrenaline.*—It depresses the response to alternating current in concentrations of 1 in  $10^4$  or greater. Lesser concentrations have insignificant effect. It increases the response to potassium and acetylcholine in concentrations 1 in  $10^7$ – $10^8$ .

*Effect of acetylcholine.*—1 in  $10^4$ – $10^5$  potentiates the response to alternating current. It causes depression if it produces tonic contraction.

*Effect of nerves.*—For some unknown reason, the muscles after one or two responses become inexcitable to nervous stimulation. The oesophagus

may become inexcitable to acetylcholine and potassium as well. The response to alternating current however remained. In such muscles the responses to alternating current did not differ significantly from other muscles. So it appears that alternating current produces its effect by direct stimulation of the muscle though the latter may also be stimulated through its nerves. It is possible however that the inexcitability may be due to lack of conduction in the nerve (Bulbring and Burn 1939) but adrenaline did not restore the responses. Potassium stimulated the duodenum which was inexcitable to nervous stimulation so it directly acts on muscle (Singh and Muthana 1946). Excess of calcium may make the muscle inexcitable to nervous stimulation but hypersensitive to acetylcholine.

#### ACTIVE ELONGATION

An interesting feature was noticed that in sodium deficient solutions the muscle elongated when stimulated with alternating current. This was produced as follows. An isometric lever was used. The hook at the bottom of the muscle chamber reached up to the narrow part of the chamber. The muscle was directly tied to the hook and it rested on the hook, it was put under slight tension of about 5-10 g. If a part of the sodium chloride (20-60%) of the saline was replaced with sucrose or lithium chloride the muscle elongated when stimulated with alternating current (8 volts). The elongation was continuation of relaxation after contraction. It occurred in 3 out of 55 experiments in duodenum in sucrose saline and in one out of 6 experiments in lithium saline and was once observed in rabbit gut in ordinary saline. It has never been produced if the muscle was placed in a trough isotonicity and then stimulated. The conditions for its occurrence are not understood. It appears that some initial tension is necessary.

The elongation may be due to two causes: (1) contraction of circular muscle, (2) active elongation of longitudinal fibres. The latter is the correct explanation as there was no evidence of marked contraction of circular muscle throughout the gut. In one experiment it was relaxed throughout. In sodium deficient solutions tone decreases so that it appears that elongation is an active process as it is in skeletal muscle (McDowall 1944, Lloyd 1946). The fact that it occurs in sodium deficient solutions supports the view that the latter may be responsible for tonus (Singh and Singh 1946).

#### SUMMARY

1. The responses of avian plain muscle in general resemble those of mammalian plain muscle.

2. Eserine has little or no potentiating effect on the action of acetylcholine

3. In sodium deficient solutions, the gut elongates activity when stimulated with alternating current

4. In a muscle inexcitable to nervous stimulation, alternating current produces its usual effects

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# CONTRIBUTIONS TO THE BIONOMICS, ANATOMY, REPRODUCTION AND DEVELOPMENT OF THE INDIAN HOUSE-GECKO, *HEMIDACTYLUS* *FLAVIVIRIDIS* RUPPEL

## Part IV The Respiratory and Vocal Organs

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	CONTENTS	PAGE
1	INTRODUCTION	57
2	TECHNIQUE	58
3	THE RESPIRATORY SYSTEM	
	(a) General	59
	(b) The Histology of the Lung	61
	(c) The Mechanism of Respiration	62
4	THE DISPOSITION OF THE PERITONEUM IN RELATION TO LUNGS	63
5	VOICE	64
6	THE SKELETON OF THE LARYNX TRACHEA AND BRONCHI	65
7	THE HYOID APPARATUS	67
8	THE SOFT PARTS OF THE LARYNX	68
9	THE MECHANISM FOR THE PRODUCTION OF VOICE	69
10	SUMMARY	70
11	BIBLIOGRAPHY	71

### 1 INTRODUCTION

THE Reptilian respiratory system has already been investigated in great detail from several standpoints—anatomical histological developmental and functional In the last century, most of the workers were naturally interested in morphological and embryological studies, while in the present one there is a perceptible preference for physiological investigations.

The anatomy and histology of the system has attracted a host of investigators since the first quarter of the nineteenth century Tiedemann (1818), Meckel (1818), Schulze (1871) Leydig (1872) Julien (1878) and Milani (1894 and 1897) studied the minute structure of the trachea and lungs; while Owen (1866) Hoffmann (1890), Gegenbaur (1899) and Wiedersheim (1890) gave a general survey of the results so achieved Wiedersheim

(1906) described the respiratory organs of the geckos *Phyllodactylus* and *Platydictylus*, and Werner (1911) those of certain rare reptiles. Abrahám (1911) investigated the nerve endings in a saurian lung, Gräper (1929) the closure of pleural cavities and the differentiation of the lung in reptiles, and Baudrimont (1929) the muscular and elastic fibres in it. Rothley (1930) dealt with the minute structure of the trachea and lungs and Smirnowsky (1930) and Dombrowski (1930) with the respiratory musculature.

The development of the lung in reptiles was worked out by Moser (1902), Hesser (1905), Bertelli (1905) and Heilmann (1914) and that of the trachea in *Lacerta agilis* by Boker (1917-18).

The physiology of the system has been the subject of numerous detailed investigations in the present century. Tornier (1904) studied the structure and function of the cervical air sacs and valves in Chameleon and Couvreur and Gautier (1904) the respiratory rhythm in it. Rainaldi (1907) described the respiratory apparatus of *Lacerta muralis*. Francois-Franck investigated the contractility and innervation of lungs in the ocellate lizard (1907) and the mechanism of respiration in *Chameleon vulgaris* (1907). Grecian Tortoise (1908) and the ocellated lizard (1907 and 1909). Baghioni (1911) gave a résumé of the comparative physiology of lung movements in Amphibia, Reptiles, Birds and Mammals. Babák (1914) dealt with the lung movements and their regulation in Lizards, Milligan (1924) with the respiration and metabolism in *Sphenodon*. Potter and Glass (1931) with the respiration in the hibernating *Phrynosoma cornutum* and Wolf (1933) with the structure and function of reptilian lungs. Gnanamuthu (1933 and 1937) tried to correlate the movements of the buccal floor with those of the thorax in lizards and turtles, while Saalfeld (1933) investigated the nerve regulation of lung movements in *Uromastix*.

The larynx of reptiles was investigated as early as 1839 by Henle in the course of his classical researches on the comparative anatomy of this organ in vertebrates. Dibois (1886), Wilder (1892) and Goppert (1899) discussed its homology. Wiedersheim (1906) described it in *Platydictylus mauritanicus*, G. rmershausen (1913) investigated it in *Chameleon*, while Schmidt (1913) studied its development in reptiles.

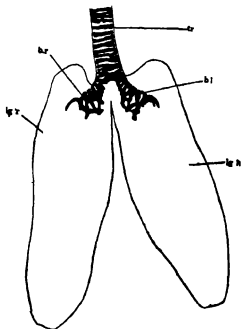
## 2 TECHNIQUE

In order to make out the relative position of the various organs in the thoracic part of the perivisceral cavity as well as to study the disposition of the peritoneum, thick hand-cut transverse sections of formaldehyde-preserved specimens, sagittal sections, and careful dissections from the right

or left side proved useful. The structure of the lungs was studied by staining small pieces in borax carmine and mounting them in balsam, as well as by preparing transverse and longitudinal sections. The skeleton of the larynx, trachea, bronchi and hyoid apparatus was investigated in entire mounts, stained either according to Dawson's Alizarin Red method, or by Van Wijhe's method of cartilage staining. The soft parts of the vocal apparatus were studied in series of transverse and longitudinal sections 10 microns thick, stained by Mallory's triple stain, as modified by Krichelsky [Stain Technology, VI (1931), 97].

### 3. THE RESPIRATORY SYSTEM

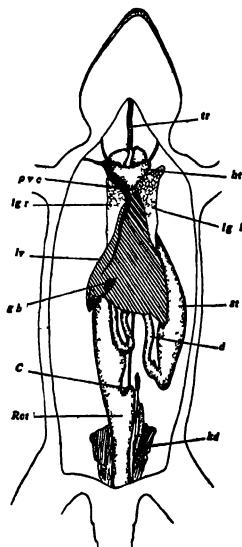
(a) *General*.—In many features, the respiratory organs of *Hemidactylus* (Text-Fig. 1) show a typical, simplified structure and appear to approximate to the condition found in *Sphenodon*. The lungs are equal and symmetrical; there are no intra-pulmonary extensions of the bronchi; the lumen of the



TEXT-FIG. 1. Respiratory Organs of *Hemidactylus flaviviridis* (from a cartilage-stained preparation).—*b.l.*, left bronchus; *b.r.*, right bronchus; *lg.r.*, right lung; *tr.*, trachea.

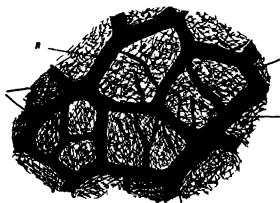
lungs is continuous and saccular, resembling that found in the Amphibia; and the respiratory surface is developed throughout, although rather less so in the posterior part. Whether these peculiarities are primitive or degenerate, is difficult to say. The glottis is a longitudinally directed, slit-like aperture,

situated on an elevation (*laryngeal prominence*) on the floor of the pharynx between the basal bifurcations of the tongue. It leads into the trachea. The latter runs ventral to the œsophagus and after traversing the cervical region comes to lie dorsal to the heart where it bifurcates into two extremely short *bronchi*. The anteriormost part of the trachea is dilated to form the larynx.



TEXT FIG. 2 General dissection, showing the various viscera—c, cecum, d, duodenum, gb, gall-bladder, ht, heart, lg l, left lung, lg r, right lung, lv, liver, kd, kidney, pvc, posterior vena cava, Ret, rectum, tr, trachea, St, stomach.

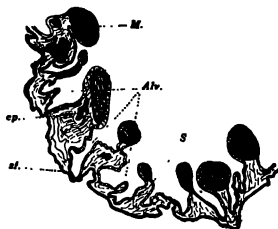
(b) *The Histology of the Lung.*—The lungs (Text-Fig. 2) are almost symmetrical structures, situated one on each side of the œsophagus, postero-lateral to the heart and lateral to the anterior half of the liver, which shows a contour particularly adapted to accommodate them. Each lung is a simple fusiform sac with thin transparent walls and complete septa inside. The wall of the posterior region of the lung is distinctly thinner than that of the anterior, and is not so richly supplied with blood capillaries. The cavity inside is continuous from one end to the other and there is no division into chambers, as found in many lizards. The inner lining is raised into a network of very delicate ridges, giving rise to a honeycomb-like appearance. The ridges are closer and more prominent in the anterior than in the posterior part, but the respiratory surface occurs throughout the entire lumen of the lungs.



TEXT-FIG. 3. Internal surface of the wall of a lung.—*Alv.*, alveoli; *R, R', R''*, inter-alveolar ridges of different sizes; *sf*, the superficial membrane.

The internal surface of each lung (Text-Fig. 3) shows a trellis-like network of ridges, which separate the *alveoli* from one another. The ridges are mainly of three sizes: one, fairly stout; the second, rather thick, though not so stout as those of the first type; and the third, extremely delicate, lying as slight elevations within the meshes formed by the ridges of the first two types. Each ridge is supported on a number of pillar-like strands, which are separated from each other by interstices and thus allow the adjacent alveoli to be in communication. The ridges and their supporting strands are covered with more or less flattened endothelial cells, and serve to increase the respiratory surface of the lung. In a transverse section (Text-Fig. 4), the inter-alveolar ridges appear as relatively large knob-like structures situated at the top of delicate, more or less crowded septa, projecting into the lumen of the lung. Each knob has a large muscle-band

inside, the shape of which differs according to the angle at which the section happens to pass through it. Muscular and fibro-elastic tissues are



TEXT-FIG. 4. Transverse section through a part of the lung.—*Alv.*, alveoli, *Cp.*, capillary; *M.*, muscle band at the distal end of the inter-alveolar ridge; *s.*, the supporting septum of the inter-alveolar ridge; *sf.*, the superficial membrane covering the lung.

also present within the supporting strands and numbers of blood capillaries can be made out, cut at various angles and interspersed in various parts of the section. The whole lung is covered by an extremely delicate superficial (serous) membrane.

(c) *The Mechanism of Respiration.*—Gnanamuthu (1933 and 1937) has given an excellent account of the mechanism of respiration in *Hemidactylus*. Not only does the 'thoracic' region expand and contract, but the posterior part of the throat also moves up and down, thereby diminishing and increasing the buccal space. As shown experimentally the buccal floor lowers when thorax dilates and rises when the chest contracts. These movements of the throat are not the passive effects of the inflation and deflation of the cavity by the entry and exit of air, but are active ones, due to the contraction and expansion of the buccal muscles.<sup>1</sup> According to Gnanamuthu (1937), the part played by the buccal floor in respiration is probably as follows:

"The contraction of the thorax expelling air would result in the inflation of the buccal cavity, and when next the thorax relaxes this impure air may be taken into the lungs again, because the thoracic contraction and expansion follow each other so rapidly. However, the elevation of the

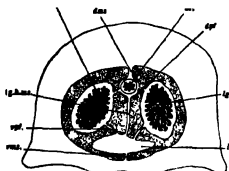
<sup>1</sup> As proved experimentally by Gnanamuthu (1937, pp. 47-48).

mouth-floor and tongue through the aid of the transverse and hyoid muscles just when the thorax contracts serves to expel the vitiated air effectively out of the body."

Whilst it cannot be denied that the buccal-floor movements may have some significance in preventing an accumulation of 'impure' air in the mouth cavity and consequently in preventing its entry again into the lungs, this sort of explanation is hardly adequate to account fully for the extremely delicate adjustments necessary for effecting an almost perfect synchronous working of the throat and the thoracic wall. Such a requirement could have been met merely by a greater development of rigidity in the buccal floor, rigidity just sufficient to cope with the extra air-pressure tending to be created in this region during expiration. What seems more likely, is, that the utility of the simultaneous movements of the throat and the thorax lies in making these two regions part of one harmonious respiratory mechanism, sucking in or forcing out air as a single structure. Exactly when the thoracic region expands, the buccal space gets also increased and the action of both in co-ordination sucks in air, as if a single chamber suddenly dilated to create a region of low air-pressure inside. Similarly their contractions synchronise to expel the foul air.

#### 4. THE DISPOSITION OF THE PERITONEUM IN RELATION TO LUNGS

Each lung (Text-Fig. 5) is suspended by two folds of the peritoneum: one, dorsal; and the other, ventral. The dorsal fold is attached to the



TEXT-FIG. 5. Transverse section of the trunk, passing through the oesophagus and lungs. *dms*, dorsal mesentery; *dps*, dorsal pulmonary fold; *g.h.ms*, gastro-hepatic region of the mesentery; *es*, oesophagus; *vms*, ventral (subhepatic) mesentery; *vpf*, ventral pulmonary fold (Other abbreviations as in text-fig. 2).

lateral aspect of the oesophagus, and the ventral (which is situated towards the inner surface of the lung) the mesial mesentery connecting the

œsophagus with the liver. Thus on either side, a recess (*pulmo-hepatic recess*, Butler; *Pneumato-enteric recess*, Goodrich) is cut off from the general body cavity between the œsophagus, lung and liver, ending blindly in front, but opening behind into the cœlom.

As a transverse partition is absent, the 'thoracic' region is not morphologically separated from the 'abdominal'. However, physiologically, the same aim is achieved in a different way. The viscera are closely packed in the 'abdominal' region and thereby indirectly delimit the 'thoracic' region in front of them. On the right side, the liver is apposed to the body-wall, while on the left the stomach lies adpressed between the liver and the body-wall. Thus the space surrounding the lungs is fairly large, while in the region posterior to them it is virtually obliterated by the closely packed viscera. This arrangement subserves to restrict the effects of the expansion or contraction of cœlom to the part immediately surrounding the lungs. Were the space surrounding the lungs in communication with a large one behind, the effects would tend to distribute themselves over a wide area and be proportionately enfeebled. As far as I know, the importance of such a disposition for respiratory activity has never been mentioned before.

### 5. VOICE

All geckos have a voice. As Smith (1935) says, "usually it is a soft chirruping or clucking sound such as we can make with our tongue, but some of the larger forms, such as *Gecko gecko*, have a loud cry that can be heard a considerable distance away; many of them squawk when captured."

The various species of *Hemidactylus* differ a great deal in their power of sound production. Some like *H. flaviviridis* produce it only rarely, and then extremely low. Others like *H. frenatus* and *H. leschenaulti* cry fairly frequently. The cry of *H. frenatus* "consists of a series of six to nine guttural sounds—*chik-chik-chik*—uttered in close succession. The cry is often very loud and clear and is a familiar voice in the vicinity of wooden fences at dusk. When caught this gecko utters a sort of feeble squeaking sound."<sup>1</sup> The voice of *H. leschenaulti* resembles that of *H. frenatus*. However, "it sometimes utters at night a prolonged shrill sound which may be expressed as *Sise-see-cek*."<sup>2</sup> *H. garnoti* has also a rather loud voice and is called, on account of it, *Tjik Tjik* in the Malayan language.<sup>3</sup>

<sup>1</sup> Smith, M. A., "Reptilia and Amphibia, Vol. II, Sauria," *Fauna Brit. Ind.*, 1935, p. 28.

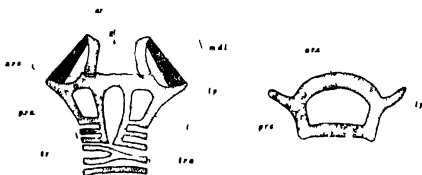
<sup>2</sup> Y. R. Rao in *St. Joseph College (Trichinopoly) Magazine*, Sept. 1915.

<sup>3</sup> Voiz, W., "Lacertilla von Palembang," *Zool. Jahrb.*, 1903, 19.

## 6 THE SKELETON OF THE LARYNX, TRACHEA AND BRONCHI

The skeleton of the air-passage was studied in seven individuals by means of van Wijhe's process of cartilage staining. As previous authors (Henle, 1839, Seck, 1908) did not have recourse to such a delicate method, the author is in a position to give a more accurate description than has been hitherto possible, as well as to add notably to our knowledge of the subject.

The wall of the *larynx* is supported by a ring-shaped *cricoid* projecting laterally to form obtuse processes and a pair of *arytenoids* fused to the antero-dorsal aspect of the cricoid on either side and bounding the glottis on the right and left sides. Gengenbaur and others held that the cricoid, arytenoids and perhaps the tracheal rings are derived from the fifth branchial arch,—a view which Goodrich (1930 p 446) criticized on the ground that the evidence is incomplete. According to Henle (1839), the larynx arises from two lateral cartilages which send transverse processes to meet each other in front of and behind the air-passage.



TEXT-FIG. 6. Dorsal view of the laryngeal skeleton—*ar*, arytenoid, *arc*, anterior ring of the cricoid, *gl*, glottis, *lc*, longitudinal connecting cartilages, *lp*, lateral process of the arytenoid, *mdl*, muscular dilator laryngis, *prc*, posterior ring of the cricoid, *tra*, tracheal rings, *tra*, abnormal tracheal ring.

TEXT-FIG. 7. Ventral view of the laryngeal skeleton—Abbreviations as in the previous figure.

The *cricoid* of *Hemidactylus flaviviridis* (Text-Figs 6-7), although composed of two successive rings as that of *Hemidactylus garnoti* (Seck, 1908) differs from the latter in many respects.

In the first place, the anterior ring in *Hemidactylus garnoti*, according to Seck (1908), is open dorsally, while the posterior ring is closed completely all round. In *Hemidactylus flaviviridis*, the anterior ring is closed dorsally as well as ventrally; while the posterior ring is open dorsally.

Secondly, the anterior ring in *Hemidactylus garnoti* is produced mesially at its antero-ventral aspect into a pointed edge which was erroneously called the processus epiglotticus, but may be more accurately designated as *Processus anterior inferior* (Göppert). In *Hemidactylus flaviviridis* the antero-ventral border of this ring is rounded and the processus anterior inferior is altogether absent.

Thirdly, there is no median projection in *H. flaviviridis* corresponding to the *processus anterior superior* of *H. garnoti*.

Fourthly, in *Hemidactylus flaviviridis* a pair of longitudinal cartilages join the dorsal part of the first ring with the dorsolateral portions of the second ring. These cartilages extend even farther back to join the first two or three tracheal rings. They are absent in *H. garnoti*; in which, however, the second, third and fourth tracheal rings are often irregularly interconnected with each other (Sack, 1908).

The arytenoids of *Hemidactylus flaviviridis* resemble those of *H. garnoti* in their attachment and direction. They articulate with the dorso-lateral parts of the cricoid at the place where the latter sweeps downwards. Each arytenoid extends forwards and inwards from its base towards its distal end, and serves to support the corresponding lip of the glottis.

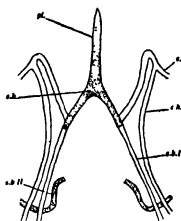
The walls of the trachea and the bronchi are supported by rings of calcified cartilage throughout their length. The number of rings in the trachea varies from 45 to 51; and in the bronchi from 6 to 8. In *Hemidactylus garnoti*, Sack (1908) found that the first 27 rings (i.e., from the 2nd to the 28th) are always completely closed, while the following ones (about 14) show a slender cleft on their dorsal surface. In *H. flaviviridis* most of the rings are closed, there being extremely few incomplete rings. Some of the rings are abnormal, as they are divided asymmetrically into anterior and posterior half-hoops.

The bronchial rings, usually 6 to 8 in number, are incomplete towards their inner sides. They are variously connected to each other to form an irregular framework, supporting the walls of the trachea. The last ring is always the largest.

Tiedemann (1818) and Meckel (1818) described a dilatation of the trachea in *Ptyodactylus fimbriatus* and regarded it as a supplementary voice-producing apparatus. In *Hemidactylus flaviviridis*, as well as in *H. garnoti* (Sack, 1908), there is no trace of such a structure.

# 7. THE HYOID APPARATUS

The hyoid apparatus (Text-Fig. 8) consists of a central portion, the *basihyoid* (*corpus hyale*), a median anterior process, the *processus lingualis* (*entoglossus*), two pairs of cornua attached to the basihyoid, the *cornua*



TEXT-FIG. 8. The Hyoid Apparatus.—*c.b. I* and *c.b. II*, first and second ceratobranchials; *c.h.*, corpus hyale; *c.h.I.* and *c.h.I'*, the two limbs of the corner hyale; *p.l.*, processus lingualis.

*hyale* and the *cornua branchialia I* (first ceratobranchials), and the vestiges of a third pair of cornua, the *cornua branchialia II* (second ceratobranchials).

The *basihyoid* is a triradiate structure lying mesially below the trachea a little behind the larynx. Its anterior median arm is continued into the base of the tongue as a slender process, the *processus lingualis* or *entoglossus*, whilst its posterior two arms, rather stouter, are movably articulated to the pair of first ceratobranchials. The *cornu hyale* of each side is attached to the basihyoid just anterior to the articulation of the latter with the first ceratobranchial. It extends forwards and outwards in a broad curve with the concavity facing backwards. Distally, it is divided into two processes: a short and straight lateral limb directed obliquely outwards, and a delicate long limb, at first projecting longitudinally backwards and then curving dorsolaterally to end underneath the paroccipital process.

The *cornu branchiale I* of each side is a well developed process, ossified throughout its extent. It curves upwards in its distal portion and ends, like the corresponding region of the cornu hyale, at the parotic region of the skull.

Gnanamuthu (1937) regarded the *cornua branchialia II* as absent in *Hemidactylus* and certain other lizards, but alizarin preparations show that

they are present as extremely slender rod-like vestiges near the distal end of the *cornu branchiale I*. Their proximal portions however are lacking.

As described above, the hyoid apparatus of *Hemidactylus flaviviridis* Ruppel is characterized by the following new features

1 The occurrence of a blunt lateral limb on the *cornu hyale* in addition to the usual prolongation reaching to the parotic region has been recorded for the first time. In certain lizards (e.g. *Mabuya*, *Cabrita* etc.) there is a small cartilaginous plate attached at this place and in *Calotes* there is a short rod-like cartilage developed here. In *Varanus* there is a peculiar crotchet-shaped cartilage connected to the proximal part of the *cornu hyale* (Gnanamuthu 1937).

2 The distal extremities of the *cornu hyale* and the *cornu branchiale I* end ventral to the paroccipital process as mentioned for *Gekkonidae*, *Uroplatidae* and *Eublepharidae* by Versluys (1936).

3 The occurrence of the distal part of the *cornu branchiale II* is remarkable. As Versluys pointed out this cornu is often divided into two parts, a proximal ventral portion and a distal dorsal portion. The former is absent and the latter present in *Hemidactylus flaviviridis*.

### 8 THE SOFT PARTS OF THE LARYNX

As in other lizards, the larynx possesses two pairs of muscles. (1) *M. compressor laryngis*, arising from the body of the hyoid and inserted on the borders of the cricoid and arytenoids and (2) *M. dilatator laryngis* arising from the lateral processes of the cricoid and inserted on the arytenoids. The former serves as a sphincter and the latter as the dilatator of the glottis.

The laryngeal muscles are supplied by two branches of the vagus nerve: an anterior one called the *Nervus laryngeus superior*, which corresponds to the first branchial branch of Fishes, and a posterior one, called the *Nervus laryngeus inferior* or *recurrens*, which represents the fourth branchial nerve and belongs to the seventh visceral arch in Fishes.

In *Hemidactylus garnoti*, according to Seck (1908), there is neither a local dilatation of the trachea as in *Ptyodactylus fimbriatus*, nor a specially wide trachea as in *Platydictylus guttatus*. There are also no vocal cords as described by other authors in the Gekkonidae. In *Hemidactylus flaviviridis*, however, the vocal cords are definitely present. They are not in the form of elastic bands stretched between the dorsal and ventral walls of the cricoid, as described for Gekkonidae by previous authors (Henle, 1939, etc.), but are prominent horizontal folds of the epithelial lining of the larynx.

(Text-Fig. 9). They lie in the anteriormost part of this chamber on the lower part of the lateral walls; and when fully extended, virtually divide an extensive dorsal space from an extremely small ventral one. The former space is supported by the arytenoids and opens at the glottis. The



TEXT-FIG. 9. Transverse section through the anterior part of the larynx, showing the vocal cords.—*d.s.*, the dorsal laryngeal space in communication with the glottis; *v.c.*, vocal cords; *v.s.*, ventral laryngeal space leading posteriorly into the trachea.

latter, when traced backwards in serial transverse sections, is seen to lead into the trachea.

#### 9. THE MECHANISM FOR THE PRODUCTION OF VOICE

According to Steck (1908), the absence of tracheal dilations and vocal cords in *Hemidactylus garnoti* is correlated with a number of anatomical peculiarities. The bases of arytenoids are shifted towards the dorsal side, so that the Ligamenta aryhoidea are more strongly developed. The dorsal borders of the arytenoids take no part in the formation of the glottis. The mucous membrane, which would in other cases cover both these cartilages as simple ridges called *Plicae arytenoideae*, is developed as a ligamentum aryricoidaeum. The glottis is abnormally large, since the first laryngeal ring is incomplete dorsally and the ligamentum aryricoidaeum extends up to the second laryngeal ring. The voice, accordingly, is produced by the vibration of the dense aryricoid ligament when it is tensely stretched and is enhanced by the floor of the mouth, which possesses a transversely striped musculature.

In *Hemidactylus flaviviridis*, however, the mechanism is different. Here, as shown by me above, the vocal cords are well developed. When fully extended, they partially separate a dorsal chamber from a ventral one. The stream of air expelled forcibly from the lungs, passes up from the ventral chamber into the dorsal one and gets out at the glottis. This sets the vocal cords into vibration and produces the sound.

## 10. SUMMARY

The author has described the respiratory and vocal organs of *Hemidactylus flaviviridis* in detail, the more important features discovered by him being as follows:—

1. The right and left lungs are equal and symmetrical, with their internal cavities undivided and saccular; the respiratory surface is developed throughout; and there are no intrapulmonary extensions of the bronchi.
2. The inner lining of the lungs is raised into a network of ridges, which are closer and more prominent in the anterior than in the posterior part. The ridges are mainly of three sizes. Their histology has been described in detail.
3. The significance of the simultaneous movements of the throat and thorax has been pointed out.
4. The disposition of the peritoneum in relation to lungs has been studied, and the rôle of the viscera in restricting the effects of the expansion or contraction of the cœlom to the part surrounding the lungs has been pointed out.
5. The *cricoid* of *Hemidactylus flaviviridis* differs from that of *H. garnoti* in the structure of its component rings, in the absence of the processes anterior inferior and anterior superior, and in the presence of a pair of dorsal longitudinal connecting cartilages.
6. The number of tracheal rings varies from 45 to 51 and of bronchial ones from 6 to 8. Most of the former are closed. The latter are incomplete towards their inner sides.
7. There is no dilatation of the trachea.
8. The hyoid apparatus is characterized by the development of a blunt lateral limb on the *cornu hyale*, by the ending of the *cornu hyale* and *cornu branchiale I* ventral to the paroccipital process, and by the presence of a vestigial *cornu branchiale II*.
9. The larynx is provided with two pairs of muscles (*M. compressor laryngis* and *M. dilatator laryngis*), which are innervated by the *Nervus laryngis superior* and the *N. laryngeus inferior*.
10. The vocal cords are present. When fully extended, they virtually separate a dorsal laryngeal chamber from a ventral one.

*The Indian House-Gecko, Hemidactylus flaviviridis Rüppel—IV 71*

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7 MAY 1947

ON THREE COCCIDIAN PARASITES *WENYONELLA*  
*MACKINNONI* N.SP., *EIMERIA LUCKNOWENSIS*  
N.SP., AND *ISOСПORA* SP., FROM THE INTESTINE  
OF THE WAGTAIL *MOTACILLA ALBA* LINN.  
(PASSERIFORMES, MOTACILLIDÆ)

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	CONTENTS	PAGE
Introduction	.. .. .	75
<i>Wenyonella mackinmoni</i> n.sp.	.. .. .	76
<i>Eimeria lucknowensis</i> n.sp.	.. .. .	81
<i>Isoспora</i> sp.	.. .. .	83
Acknowledgments	.. .. .	85
References	.. .. .	85

INTRODUCTION

DURING the winter of 1940, eight specimens of the common wagtail *Motacilla alba* Linn. were entrapped in Lucknow and an examination of their droppings revealed a coccidial infection in two out of eight birds. In order to study the exogenous stages of development of this coccidian, the droppings as well as the rectal contents of the infected birds after dissection were kept in 1 per cent. solution of chromic acid. Each oocyst, after sporulation, showed four sporocysts inside it, and each sporocyst in turn had four sporozoites, i.e., four sporocysts and sixteen sporozoites were present in each oocyst—a diagnostic character of the genus *Wenyonella* Hoare, 1933.

DURING the winter of 1941, six more specimens of the same bird were examined for coccidial infection; out of these five proved to be coccidia-free, but one was passing two kinds of oocysts along with its faeces: (i) oval oocysts, which were colourless, and (ii) spherical oocysts with thick yellowish inner cyst walls. In 1 per cent. solution of chromic acid, after complete sporulation, these oocysts were diagnosed as belonging to the genera *Eimeria* Schneider, 1875, and *Isoспora* Schneider, 1881, respectively.

The coccidia-free specimens of *Motacilla alba* could not be infected artificially, as they died, (probably they could not stand confinement for long), before the oocysts of the three above-mentioned coccidian parasites could sporulate and attain the infective stage in the culture medium.

Pieces of small intestine were fixed in Bouin-Duboscq-Brazil, sectioned  $4-6\mu$  thick, and stained with iron-alum haematoxylin and chromotrope 2 R, or Davidson's haematoxylin only. A few fresh smears of the scrapings of the intestine were made in normal saline solution and examined under an oil-immersion lens, but no motile stages of the parasites could be detected. Similar smears fixed in Schaudinn's fluid and stained with iron-haematoxylin were also examined but they did not yield any significant result besides those that had been obtained from a study of the sections of the intestine.

It may be mentioned here that only six species of *Wenyonella* have been recorded up to date (*vide* Table I). However, the species of *Wenyonella* described in this paper differs from those mentioned above in certain particulars, and therefore, I propose to designate this coccidian of the wagtail as *Wenyonella mackinnoni* n.sp., the specific name being given in honour of Prof. Doris L. Mackinnon of the King's College, London.

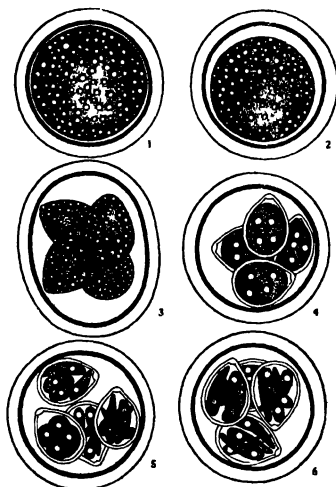
*Wenyonella mackinnoni* n.sp.

**Exogenous stages.**—The oocysts are spherical or ovoid in shape; they measure  $19\mu-23\mu$  in spherical forms, and  $23.8\mu-26.2\mu \times 18.0\mu-21.5\mu$  in ovoid forms. The cyst wall consists of two layers: an outer layer which is thin and colourless, and an inner layer which is comparatively thicker and is brownish in colour. The protoplasm of the freshly discharged oocyst is filled with refractile granules of reserve materials and occupies the entire internal space (Text-Fig. I, 1), but later on it becomes condensed and has a more or less spherical contour and measures, on an average,  $15.5\mu$ , in diameter (Text-Fig. I, 2); the micropyle and the polar inclusions are absent in the oocysts.

Oocysts kept in 1 per cent. solution of chromic acid and examined at regular intervals of six hours at room temperature revealed visible signs of segmentation of the zygotes within 24 to 36 hours, and within next 48 to 60 hours four rounded bodies, the sporozoites, were cleaved out of the protoplasmic bulk of the zygote. Usually the sporoblasts remain adhering together for some time, but later on they separate (Text-Fig. I, 3), become ovoid, and each of them secretes a wall around itself and thus forms the sporocyst (Text-Fig. I, 4). There is no oocystic residue left after the formation of the sporocysts. Each sporocyst measures  $10.2\mu \times 7.4\mu$  in

*Three Coccidian Parasites from Intestine of Wagtail M. alba Linn. 77*

size, and has a lens-shaped thickening at its narrower end. The protoplasm of the sporocyst in turn segments without leaving any residue, into four rounded bodies the precursors of the sporozoites which later on elongate ( $8.2\mu$  long) and assume a club-shaped appearance (Tex-Fig I



(All figures were drawn with the aid of camera lucida)

TEXT-FIG. I.—Showing exogenous stages of *Wenyonella mackinnoni* n. sp.

From living specimens  $\times 1900$

1, a freshly discharged oocyst 2 oocyst with unsegmented but condensed zygote 3, oocyst showing formation of sporoblasts 4, oocyst with four sporocysts 5, 6, oocysts showing sporocysts, each with four sporozoites

5, 6). They are arranged at random inside the sporocysts. The formation of the sporozoites takes place during the next 24 to 48 hours after the formation of the sporocysts, i.e., complete sporulation takes 4 to 6 days

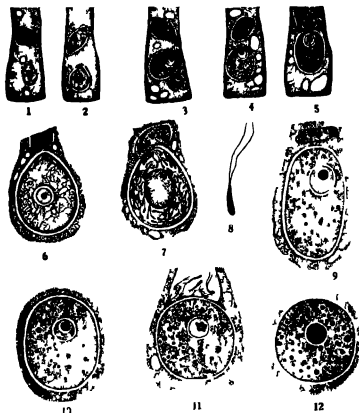
**Endogenous stages**—The endogenous cycle of development takes place in the small intestine of the host. It may be mentioned at once that no asexual or schizogonic stages of the parasite could be detected, and the only stages frequently encountered were (i) the microgametocytes and microgametes, (ii) the macrogametocytes and macrogametes, and (iii) the zygotes. On one occasion only a few microgametes were noticed within the cavity inside an epithelial cell lodging a macrogamete (Text-Fig II, 11), but no stage showing the entrance of a microgamete into the macrogamete could be encountered. Besides these stages, young developmental stages of the sexual forms measuring  $2.2\text{--}6.0\text{ }\mu$  were also seen on certain occasions (Text-Fig II 1-5). They have been designated as the sexual forms following Ray and Das Gupta's (1937) view of distinguishing the schizonts from the sexual forms of *W. hoare* and also because they exhibit resemblances to the mature sexual forms in their cytoplasmic and nuclear contents.

The entire absence of schizogonic cycle may be due to the fact that it was over when the birds were examined. It seems that this parasite, like other coccidia, also undergoes a course of "self-limited" infection.

The grown-up microgametocytes (Text-Fig II, 6) measure, on an average,  $20.5\text{ }\mu \times 15.6\text{ }\mu$  in size and can be distinguished from the macrogametocytes, besides their size, by having (i) a conical shape, (ii) a ragged cytoplasm, and (iii) a centrally located nucleus with a centrally situated karyosome which is comparatively smaller than that of the macrogametocyte. A mature microgametocyte gives rise to several microgametes (Text-Fig II, 7), leaving a considerable bulk of cytoplasm unused. Each microgamete (Text-Fig II, 8) has an elongated body ( $3.8\text{ }\mu$  long) and two equal flagella which are nearly twice the length of the body. Whether the flagella are attached anteriorly or posteriorly is difficult to say, because the movements of the microgametes could not be observed *in vivo*.

The grown-up macrogametocytes (Text-Fig II 9) are ellipsoidal bodies, rounded at both ends, and measure, on an average  $28.5\text{ }\mu \times 16.0\text{ }\mu$  in size. The cytoplasm of each macrogametocyte contains reserve materials and the nucleus lies rather nearer the superior pole. The karyosome is fairly big and excentric in position, being surrounded by a clear space. During the course of its development the macrogametocyte becomes more or less globular in shape, and gives rise to a single macrogamete (Text-Fig II, 10, 11) measuring  $23.0\text{ }\mu \times 19.8\text{ }\mu$  in size. On no occasion could a micropyle be detected in the macrogametes of *Wenyonia mackinnoni* (cf. *W. hoare*; Ray and Das Gupta, which possesses a prominent micropyle).

The zygotes (Text-Fig II 12) can be distinguished from the macrogametocytes and macrogametes by having (i) a denser accumulation of reserve materials (the so-called plastic and hæmatoxylinophilic granules) and (ii) a homogeneously stained nucleus



TEXT FIG. II.—Showing endogenous stages of *Wenyonella mackinnoni* n. sp.  
From sections of small intestine

Figs 1-5 Developing sexual forms  $\times 1750$  1, 2 microgametocytes 3, 4, 5 macrogametocytes, in fig 3 two parasites are seen in a single cell. Figs 6-12 Mature sexual forms 6, a microgametocyte  $\times 1000$  7 showing several microgametes and a central 'restkörper' (semi-diagrammatic)  $\times 1000$  8 a highly magnified microgamete  $\times 2500$  9, a macrogametocyte  $\times 1050$  10, 11 macrogametes in fig 11 a few macrogametes are seen lying near the macrogamete  $\times 1050$  12 a zygote  $\times 1050$

**Diagnosis.**—Tetrazoic tetrasporocystid condition of the oocysts determines the position of this coccidian under the genus *Wenyonella* Hoare, 1933

Oocysts spherical or ovoid, measuring  $19.0\mu-26.2\mu \times 18.0\mu-21.5\mu$ ; cyst wall thick, double-layered, outer colourless, inner brownish, micropyle absent; sporocysts ovoid, measuring  $10.2\mu \times 7.4\mu$ , with a lens-shaped

TABLE I  
Comparison between the different species of *Wentvonella*  
The measurements are given in microns

Name	Oocyst		Sporulation period	Sporocyst		Host	Habitat	Locality
	Shape	Measurements		Shape	Measurements			
1 <i>W. africana</i> Haeckel, 1933	Subspherical or ovoid	18.5-19.2 x 16.0-17.6	Absent	Ovoid lensiform present at the pole narrower	9.6 x 8.0	<i>Boscanus</i> sp. Opilidia Reptilia	Subepithelial tissues of small intestine	Etsebe, Africa
2 <i>W. bovis</i> Ray and Das Gupta, 1935	Spherical	14.9-18.5	Do	Do	10.0 x 8.0	<i>Sarcos</i> sp. (Rodentia Mammalia)	Epithelium of small intestine	Calcutta, India
3 <i>W. ulmani</i> Berghe, 1938	Ovoid	26.0-30.0 x 19.0-20.0	Transient	Ovoid, both ends similar lensiform knob absent	11.4 x 7.6	<i>Funisoma</i> <i>enrydina</i> (Rodentia Mammalia)	Probably the intestine	Belgian Congo Africa
4 <i>W. ferris</i> Berghe, 1938	Subspherical	16.2-18.3	Absent	Do	7.6 x 5.0	<i>Tramys</i> (Rodentia Mammalia)	Do	Do
5 <i>W. bali</i> Misra, 1944	Sub-spherical or ovoid	16.0-17.5 x 14.6-15.5	Do	Egg shaped lensiform knob absent	6.6 x 4.2	<i>Citellus</i> (Rodentia Mammalia)	Small intestine	Lucknow India
6 <i>W. gallinae</i> Ray, 1945	Oval or egg shaped	29.48 x 23.50 x 19.84 x 22.78	Do	Short necked rounded bottomed ovals	18.76 x 8.04	<i>Gallus gallus domesticus</i> (Galliformes Aves)	Epithelium of the terminal part of the intestine	Matherwar, Kanan, India
7 <i>W. macdonnensis</i> n. sp.	Spherical or ovoid	19.0-26.2 x 18.0-21.5	Absent	Ovoid lensiform knob present at narrower pole	10.2 x 7.4	<i>Meleagris</i> (Passeriformes Aves)	Epithelium of small intestine	Lucknow India

*Three Coccidian Parasites from Intestine of Wagtail Motacilla alba Linn.* 81

knob at one end; sporozoites 8.2  $\mu$  long club-shaped irregularly arranged, oocystic and sporocystic residual bodies absent, sporulation time 4 to 6 days; unsegmented oocysts discharged in the faeces of the host

*Systematic position*—*Wanyonella mackinnoni* n sp (Eimeriidae Cocciduda)

*Habitat*—Small intestine of *Motacilla alba* Linn

*Locality*—Lucknow UP India

The accompanying table shows a comparison of the known species of *Wanyonella* with regard to oocysts sporocysts hosts etc

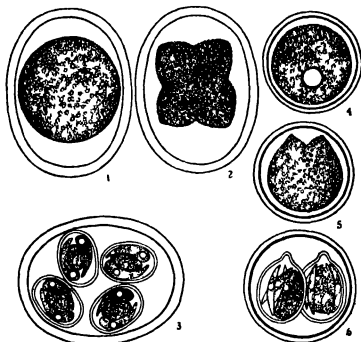
*Eimeria lucknowensis* n sp

To the best knowledge of the author there is only one species of *Eimeria* namely *E. roscoviensis* (Labbé 1893)\* recorded from *Motacilla alba* Linn. This parasite has, however been reported from other birds as well e.g. *Phalacrocorax aristotelis* *Charadrius cantianus* *Streptopelia interpres* *Numenius phaeopus* *Pulvialis apricarius* *Totanus calidris* etc (vide Levine and Becker 1933). Labbé in his previous paper (1893) did not give any illustration of the oocysts of *E. roscoviensis* but in a later contribution (1896) he supplemented a figure (vide his Pl XVII fig 18) of the mature oocyst of this coccidian which gives a clear idea of the structure of its oocyst. A comparison of the mature oocyst of the species of *Eimeria* described in this paper with that of *E. roscoviensis* as sketched by Labbé would at once reveal that the latter species does not coincide in its characters with the former and therefore a new specific name, *Eimeria lucknowensis* n sp has been instituted for the present coccidian.

The oocysts of *E. lucknowensis* are ovoid in shape and are eliminated in an unsegmented condition in the faeces, they measure 21.4  $\mu$ –24.5  $\mu$   $\times$  17.4  $\mu$ –18.8  $\mu$  in size. The cyst wall is colourless and double layered, both ends of the oocysts are similar and rounded and there is no indication of a flattening or prolongation at either end nor is there any evidence of a micropyle and polar inclusions. In these characters *E. lucknowensis* differs markedly from *E. roscoviensis* in which the oocysts are pyriform in shape, measure 16.0  $\mu$ –18.0  $\mu$   $\times$  14.0  $\mu$ –16.0  $\mu$  and each mature oocyst is characterized by the presence of a truncated neck bearing a pseudo-micropyle, as well as the polar globules. In 1 per cent solution of chromic acid the oocysts of *E. lucknowensis* sporulate within 3 to 4 days. There is no residual body inside the oocyst after the formation of the sporoblasts.

\* Labbé in his original paper (1893 p 408) has named it as *Coccidium roscovianus*

which are four in number, and which later on secrete a wall around each one of them, thus giving rise to the same number of sporocysts (Text-Fig III 1-3). Each sporocyst is ovoid in shape and is devoid of any thickening



TEXT-FIG III—Showing sporulation in *Elmeria lucknowensis* n sp and *Isospora* sp  
From living specimens  $\times 1750$

1, oocyst of *E. lucknowensis* n sp with unsegmented zygote 2 showing formation of sporoblasts 3 an infective oocyst with four sporocysts each with two sporozoites and a residual body 4 a freshly voided oocyst of *Isospora* sp showing formation of two sporoblasts 5 an infective oocyst with two sporocysts, each containing four sporozoites and a voluminous residual body

at either pole, it measures  $8.5 \mu \times 6.0 \mu$ . The end-product of sporogony is the formation of two club-shaped, curved sporozoites within each sporocyst, the sporozoites measure  $7.0 \mu$  in length and are arranged with their concavities facing the sporocystic residuum between them. The sporocyst of *E. roscoffensis*, on the other hand, is pyriform in shape, and has a knob-like thickening at its narrower pole; moreover, the two sporozoites in each sporocyst lie on one side, the other side of the sporocyst being occupied by the residual body.

A study of the endogenous stages found in the small intestine was not conclusive, because of the simultaneous presence of the endogenous stages of another coccidium *Isospora* sp. described below.

*Three Coccidian Parasites from Intestine of Wagtail M. alba Linn.* 83

**Diagnosis**—Dzoic tetrasporocystid condition of the oocysts places this coccidian under the genus *Eimeria* Schneider, 1875

Oocysts ovoid,  $21.4\mu-24.5\mu \times 17.4\mu-18.8\mu$ , discharged unsegmented in the faeces, sporocysts ovoid,  $8.5\mu \times 6.0\mu$ , sporocystic residue in between the two sporozoites, sporulation period 3 to 4 days

**Systematic position**—*Eimeria lucknowensis* n sp (Eimeriidae, Coccididae)

**Habitat**—Small intestine of *Motacilla alba* Linn

**Locality**—Lucknow, U.P., India

*Isospora* sp

Only one coccidian belonging to the genus *Isospora* namely, *I. passerum*\* Sjöbring, 1897, has been reported from *Motacilla alba* Linn. This parasite, however, has been recognised as a synonym of *Isospora lacazei* (= *Diplospora lacazei*) Labbé, 1893, of the passerine birds and held by certain workers to be a pathogenic species. Thus Labbé (1893) mentioned that *I. lacazei* proved fatal to finches infected experimentally with sufficient doses of this parasite. Hadley (1910) asserted that the common English sparrow and other birds, if chanced to find access into the poultry runs, could transmit white diarrhoea to young fowls and blackhead to turkeys, the causative agent being the same parasite. Becker (1934) stated that this "parasite has a special interest because it is a cause of loss among caged birds, particularly canaries."

Existence of more than one species of *Isospora* in passerine birds has been suggested by several protozoologists, e.g. Latta (1893), Wenyon (1926), Becker (1934), etc. but cross-infection experiments have not been conducted to support their views. Labbé (1893), however recorded *Isospora rivolta* (= *Diplospora rivolta*) from chaffinch, speckled magpie and mouse (all passerines), the distinguishing characters of this coccidian being the comparatively heavier wall of its oocysts and the oocysts required not less than 15 days (Labbé in 1896 mentions "douze a quinze jours") for development, whereas in *I. lacazei* the walls of the oocysts are thinner, and the oocysts required 4 to 5 days (Labbé in 1896 mentions "trois ou quatre jours") for sporulation. Although Labbé has given no illustrations of *I. rivolta*, the above-mentioned differences, as well as the differences in the measurements of the oocysts (in *I. lacazei*  $23\mu-25\mu$  and in *I. rivolta*  $16\mu-18\mu$ ) are,

\* Also known as *Isospora communis passerum* Sjöbring, 1897

† Hadley's announcement of the pathogenicity of this parasite has, from cross-infection experiments, been proved untenable by Smith and Smilie (1917), Johnson (1923), Boughton (1929), etc., since all attempts to infect fowls with this coccidian have met with failure.

I think, quite suggestive of regarding these two parasites as distinct and separate species.\* Bøcker (1934, p. 101) has also expressed that the species of *I. rivolta*, 'as well as some new ones, may have to be recognised'. However, the present species of *Isospora* differs in certain respects from *I. lacazei* but in the size of its oocysts, comparatively thicker cyst walls, and delayed period of sporulation, it approximates to *I. rivolta*, and therefore, it has been avoided to dub a new specific name to it. The distinguishing characters of this coccidian are given below.

The oocysts are spherical,  $14.8\mu-17.8\mu$  in diameter, and are discharged in an unsegmented condition along with the faeces of the host, the cyst-wall is two-layered, the inner layer being comparatively thick and yellowish in colour, while the outer one is thin and colourless, micropyle and polar inclusions are absent; sporulation (Text-Fig. III, 4-6) in 1 per cent solution of chromic acid requires 10 to 12 days, two sporocysts are formed in each oocyst and the oocystic residue is absent. Each sporocyst, measuring  $10.6\mu \times 7.4\mu$ , is ovoid in shape having one pole rounded and the other narrower, the latter having a nipple-like knob at its extremity, the Steida body is invariably absent in the sporocyst. The contents of the sporocysts undergo segmentation and thus four spindle-shaped sporozoites measuring  $7.5\mu$  in length are formed, and a voluminous residue is left inside each sporocyst. The arrangement of the sporozoites does not follow any regular order.

Biogenous stages were not conclusive due to a mixed infection (*vide supra*).

**Diagnosis**—Tetrazoic disporocystid condition of the oocysts locates this coccidian under the genus *Isospora* Schneider, 1881.

Oocysts spherical,  $14.8\mu-17.8\mu$ , unsegmented in fresh faeces; sporocysts ovoid, with nipple-like knob,  $10.6\mu \times 7.4\mu$ , sporulation time 10 to 12 days.

**Systematic position**—*Isospora* sp. (Eimeridae, Coccidiida)

**Habitat**—Small intestine of *Motacilla alba* Linn.

**Locality**—Lucknow, U.P., India.

\* The coccidian *I. rivolta* Grassi, 1879, which inhabits the intestines of cats and dogs, has been mentioned as *I. rivolta* by certain writers, e.g. Leuckart (1886, p. 221, *Coccidium rivolta*), Dobell and O'Connor (1921, p. 98), etc. If *I. rivolta* (Labbé, 1893) is recognised as a valid species, it is suggested, in order to avoid confusion between the two different parasites—the one occurring in cats and dogs and the other in birds that the name *I. rivolta* should be substituted by *I. labbei*, a n.

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# STUDIES ON THE REFRACTIVE INDEX OF MILK

## Part I Observations on Genuine Samples

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VARIOUS official tests, physical and chemical, have been devised for the determination of added water in milk. The principal ones are the well-known presumptive standards for fat and solids not fat of milk, the cryoscopic test (Beckmann, 1894) and the refractive index of the sour (Leach and Lythgoe, 1903), acetic (Leach and Lythgoe, 1904) and copper sulphate serum (1910) of milk, prepared under standard conditions. The preparation of the milk-serum has been resorted to owing to the opacity of milk when viewed through the immersion refractometer. The time-consuming chemical procedure of this method has brought about the popularity of the cryoscopic test, although the latter needs a considerable amount of skilled technique in its measurement.

In this paper standardisation of a simple and quick method of determining the refractive index of milk, in contradistinction to that of milk-serum, with the Abbe Refractometer has been attempted. The cow and buffalo being equally common milch animals in India, the range of variation of R.I. for each type of milk has been studied. Further, the relationship between the density and the R.I. (*i.e.* Refractive constant,  $K$ ) have also been calculated for a large number of samples. The data from all these determinations have been statistically analysed.

### EXPERIMENTAL

The R.I. determinations with the Abbe Refractometer were made, for the first few samples of milk, on whole milk. But it was found that the presence of fat in milk necessitated a very quick adjustment of the total line of reflection in the refractometer, as otherwise the line tended to blur and flow with delay. Defatted milk, therefore, gave a sharper and more permanent line of demarcation without affecting the measure of the R.I., thus permitting a greater degree of accuracy and freedom in the determination. Table I illustrates this fact.

TABLE I  
*R.I. (40° C) of whole and skimmed milk*

Cow		Buffalo	
Whole	Skimmed	Whole	Skimmed
1.3450	1.3451	1.3470	1.3472
65	66	68	68
62	61	61	60
65	64	77	76

After a few trials the following method was finally adopted 10 c.c. of sample is pipetted into a Gerber butyrometer and centrifuged for 5 minutes when almost all the fat forms a plug on top leaving skimmed milk at bottom. A few c.c. of the latter is carefully collected in a test-tube without disturbing the disposition of the two layers, and the R.I. determined on the skim milk. The readings were taken when the temperature of the instrument was steady at 40° C, and repeated with fresh drops until the difference between consecutive readings did not exceed 0.0003.

Samples of milk for examination were mostly obtained from the Military Dairy Farm, Hebbal, about 3 miles from the Institute. About 1 to 2.5 hours lapsed between milking and the analysis of the samples, the time lapse causing no detectable difference in the R.I.

The farm has about 400 milking animals, the cows belonging to Scindhi, Tharparkar, Ongole, Cross (Ayrshire × Indian) and C.P. breeds, and the buffaloes to Delhi, Nagpur and Neeli breeds. Samples were collected both from individual animals as well as pooled milk, both chosen, as far as possible, at random from the herd. The bulk samples were collected from cans containing the yield of 15–25 animals. About half the number of cows were suckled by calves before milking, but the buffaloes were all milked without this practice. The animals were in all stages of lactation from 15 days to about 8 months after parturition. Samples were collected in the morning (9 to 11 in summer and 7 to 8 in the rainy season) by the laboratory attendant, in whose presence the animals were milked, and brought to the laboratory in sealed cans. The acidity of the samples lay between 0.09 and 0.11 per cent. Thus the data analysed in this paper cover a period of about 8 months, from March to June (dry summer) and July to end of September (rainy season) when plenty of green pasture is available.

The animals in the dairy farm being managed under standard conditions, it was thought advisable to test random samples from animals under widely differing conditions of management. The City of Bangalore is largely supplied by producers who own hardly a few animals each. Animals in the City are stall-fed, while those in nearby villages go out to pasture. While rich owners feed their cattle with concentrates like cottonseed, groundnut cake, etc. poorer ones supply mostly hay and grass, and perhaps a little rice bran. Thus, about 30 samples each of cow and buffalo milk were collected at random from all classes of owners for examination.

The composition of a large number of samples collected were also estimated. The density (Celsius lactometer  $20^{\circ}\text{C}$ ) and fat content (Gerber process) were used for computing the total solids (which closely agreed with the values of actual estimation) with the following formula for Indian milk

$$TS = 0.25 (D-1000) + 1.2 F + 0.66$$

The Refractive constant  $K$ , has been calculated according to the Lorenz and Lorentz formula

$$\frac{n^2 - 1}{n^2 + 2} \times \frac{1}{d} = K,$$

where  $n = RI$  ( $40^{\circ}\text{C}$ ),  $d = \text{density}$  ( $20^{\circ}\text{C}$ ) of milk

More than 200 samples each of cow and buffalo milk have thus been analysed. The frequency distribution of  $RI$  and  $K$  are represented in Figures 1 and 2.

The relationship between  $SNF$  and  $RI$  and between  $SNF$  and  $K$  are illustrated in Figures 3 and 4.

#### DISCUSSION OF DATA

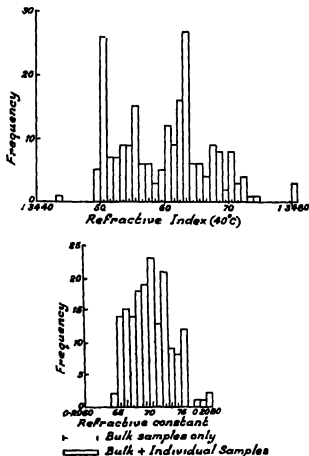
##### *Effect of Defatting Milk on RI —*

The figures in Table I show that removal of fat by centrifuging causes no difference in  $RI$  of milk. This is to be expected as the fat is only a suspension in milk and forms no part of the solutes which contribute to the  $RI$ .

##### *Limits of RI and K of Milk —*

While it is realised that a much larger number of analyses are to be completed before studying statistically the nature of the frequency curve, it is felt that the data collected so far are enough to warrant the fixing, at

least tentatively of the extreme limits of variation of R I and K for genuine cow and buffalo milk



**Fig 1 Frequency Distribution of Refractive Index and Refractive Constant of Cow Milk**

Of all the samples examined about 50 per cent were made up of individual samples and the rest of bulk samples. Among these about half were analysed in the dry months March to June and the rest in the months July to October when lush vegetation was available for cattle.

It is seen from Figs 12 that the limits of R I of individual samples of cow milk normally extend from 1.3449 to 1.3480 buffalo milk from 1.3461 to 1.3500. The limits are however considerably narrowed down with bulk samples due to the ironing out of extremes of individuality by pooling milk. For cow milk these are 1.3450 to 1.3471, and for buffalo milk 1.3462

to 1.3487. It may here be repeated that these figures are inclusive of variations due to season, individuality and type of management (farm or village-bred cattle) and composition (cow milk, fat 2.15-7.1, total solids 11.47-16.00, S.N.F. 7.97-9.35 and ash 0.66-0.75 per cent.; buffalo milk, fat

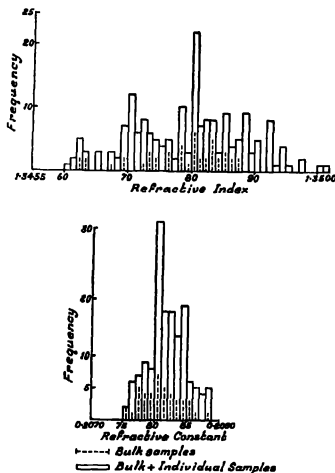


Fig. 2 Frequency Distribution of Refractive Index and Refractive Constant of Buffalo Milk

5.0-11.0, total solids 13.8-20.0, S.N.F. 8.03-11.3 and ash 0.70-0.85 per cent.). Within these limits the most frequently distributed value (the mode) is 1.3463 for cow and 1.3480 for buffalo milk. But it will be noticed that there are secondary maxima in each of the frequency diagrams. Analysis of the data (which are not given in detail due to shortage of space) in the light of seasonal variations indicate that the R.I. in the dry months has

an average and a mode distinctly lower than in the months when a plentiful supply of green herbage is available for consumption. Thus the mode is 1.3450 for cow and 1.3470 for buffalo milk in summer.

**Refractive Constant.**—The frequency diagram of this constant brings out the fact that the range of variation of the constant is not only considerably narrower than that of R.I. but is much less subject to changes due to external factors. For cow milk, K normally ranges from 0.2065 to 0.2075, and for buffalo milk from 0.2076 to 0.2088. The modes, 0.2070 for cow and 0.2080 for buffalo milk are also quite distinct from each other. Further, the range of K, unlike that of R.I., is practically the same for both individual and bulk samples, which is an added advantage.

*Relationship between R.I. and K in cow and buffalo milk —*

It is noteworthy that although a certain degree of overlapping occurs in the ranges of R.I. of the two types of milk, K is characteristically different for each milk in the overlapping range. Table II brings out this fact clearly.

TABLE II  
*Relationship between R.I. and K in cow and buffalo milk*

Cow		Buffalo	
Range of R.I. (40° C.)	K	Range of R.I. (40° C.)	K
1.3440-60	0.2065	1.3462-479	0.2076
49-60	66	67-80	77
49-63	67	66-86	78
50-66	68	70-87	79
54-73	69	63-92	80
49-67	70	71-93	81
53-69	71	72-94	82
56-70	72	78-97	83
54-72	73	72-501	84
62-70	74	82-494	85
43-80	75	84-92	86
72-78	76	80-97	87
	79	90-94	88

With the help of K and the corresponding R.I. it is thus possible to characterise the type of milk under examination with considerable certainty.

*Relationship between S.N.F. and R.I. and K of Milk.—*

Fig. 3 illustrates the approximate relationship between R.I. and S.N.F. of milk. Gross differences in S.N.F. are reflected, more or less, in corresponding changes of R.I. Elsdon and Stubbs (1929) observed a similar

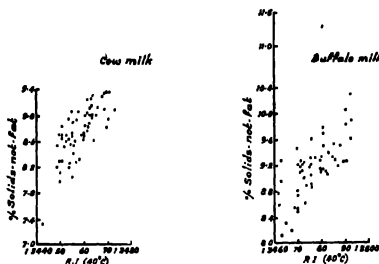


Fig.3. Relationship between Solids-not fat and Refractive Index of Milk.

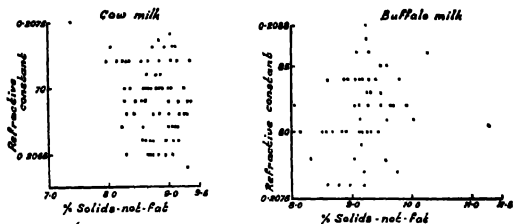


Fig.4 Relationship between S.N.F. and K in Cow and Buffalo milk.

relationship between S.N.F. of milk and R.I. of milk-serum. But it is of advantage that no such relationship (Fig. 4) exists between S.N.F. and K. The figure shows that in spite of abnormal changes in the S.N.F. content of milk, K remains within normal limits.

TABLE III

*Relationship between certain constants of milk low in S.N.F.*

Density (20° C.)	S N F. %	R. I. (40° C.)	K
Cow Milk			
1.0272	8.38	1.3457	0.2070
47	7.97	50	72
48	8.14	50	72
73	8.22	49	67
56	8.23	54	72
69	8.30	50	68
73	8.28	55	70
81	8.30	50	65
15	7.36	43	75
50	8.29	51	72
80	8.43	56	68
82	8.32	57	72
85	8.04	54	73
73	8.28	53	69
Buffalo Milk			
1.0280	8.31	1.3465	0.2078
85	8.04	78	82
44	8.13	63	80
40	8.19	67	83

It can be seen from the above figures that samples of milk low in S.N.F. (with less than 8.5%) are usually associated with low R.I. and values of K considerably above the minimum for normal milk. It is perhaps possible that such a correspondence of the two constants is, as a rule, characteristic of genuine samples with low S.N.F. content

In the light of the above data it is concluded that samples of cow milk with R.I. less than 1.3449 and K less than 0.2065 and buffalo milk with R.I. less than 1.3461 and K less than 0.2076 can be considered to be adulterated.

## SUMMARY

A simple method of determining the refractive index of milk with the Abbé refractometer has been devised. About 10 c.c. of sample in a Gerber butyrometer is centrifuged for 5 minutes in an ordinary milk centrifuge. A few c.c. of the defatted milk is carefully collected without disturbing the fat layer and tested for R.I. It takes less than 30 minutes to test the R.I. of a dozen samples in this fashion; and these values represent, unlike those of milk-sera, the true refractive index of milk. From the density and R.I. the refractive constant, K, has been calculated.

The R I and K of more than 200 samples each of cow and buffalo milk have been tested over a period of 8 months. The limits of R I for cow milk lie between 1 3449 and 1 4480 the mode being 1 3463 and for buffalo milk between 1 3461 and 1 3500 the mode being 1 3480. K is distinct and lies within much narrower limits for each type of milk cow milk 0 2065-0 2075 and buffalo milk 0 2076-0 2088. These limits unlike those of R I are independent of the solids not fat content of milk. From the available data it is concluded that samples of cow milk with R I 1 3449 and K 0 2065, and buffalo milk with R I 1 3461 and K < 0 2076 can be considered to be adulterated.

#### ACKNOWLEDGMENT

I am indebted to Mrs P Rangappa B Sc for the statistical analysis of the data.

My thanks are due to Mr B N Banerjee and Prof V Subrahmanyam for their kind interest.

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# ON *BEANIOPSIS RAJMAHALENSIS* GEN. ET SP. NOV., A NEW TYPE OF GYMNOSPERM FEMALE FRUCTIFICATIONS FROM THE JURASSIC OF BEHAR

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CONTENTS	PAGE
INTRODUCTION	95
DESCRIPTION	96
Generic diagnosis	96
Specific diagnosis	97
Cone axis	98
Sporophyll stalk	98
Seeds	99
COMPARISONS	100
SUMMARY	102
BIBLIOGRAPHY	103
EXPLANATION OF PLATES	103

## INTRODUCTION

THE material on which this paper is based was collected at Onthea, during an excursion with Professor Birbal Sahni, F.R.S., in January 1942. The village of Onthea (in the Province of Behar) is situated at a distance of about 4 miles due south of Maharajpur railway station on the E.I.R. loop line between Sahebganj and Tinpahar (Ganju, 1946, Text-fig. 1). Most of the forms described here were discovered on breaking hard siliceous rocks which apparently showed nothing on the exposed surfaces. The work was carried out at the Lucknow University under the guidance of Prof. B. Sahni. The figured material is preserved in the museum of the Department of Botany and Geology.

I wish to express my deep sense of gratitude to Prof. Sahni for his constant help and guidance given me readily throughout this work. I am greatly indebted to him for spending a good deal of his valuable time with me in discussing the nature and affinities of the new forms described in this and the two succeeding papers which will be published shortly. My thanks are also due to Mr. R. N. Lakhanpal, M.Sc., for kindly correcting the proofs of this paper.

This work records the find of a very interesting new type of Gymnosperm seed-bearing fructification resembling in some respects the genus *Beania* from the Jurassic of Yorkshire which is now regarded as the fructification of a true cycad but having no clear affinity with that genus.



TEXT FIG. 1 *Beanopsis rajmahalen* gen. et sp. nov. showing portion of the cone axis and a pair of seeds lying near it. The axis is longitudinally striated. On the left of the axis a sporophyll stalk is seen to arise. The two seeds are flattened on the base of each a distinctly rounded and cordate and the micropylar end is elongated and flattened giving the entire seed a rather flask-like form. O 18 C 12½.

TEXT FIG. 2 *Beanopsis rajmahalen* is gen. et sp. nov. Two pairs of seeds lying near each other. Each seed shows a very distinct median longitudinal groove continued from its base to apex. The micropylar ends of the two seeds of a pair point in a direction away from each other. O 19 C 20.

#### DESCRIPTION

#### *Beanopsis* gen. nov.

#### Generic Diagnosis

*Female gymnospermous strobili of obscure affinity resembling Beania in their broad features but differing in the fact that each sporophyll is expanded*

*distally into a spoon-like organ which bears upon its upper surface two divergent platyspermic ovules*

This genus is based upon seven well preserved specimens, all from Onthea. The specimens contain well preserved sporophylls attached to a central axis. The scales are stalked and each bears two ovoid sessile seeds placed distally in a broadly U-shaped depression of the sporophyll.

As regards the affinities, this genus is obviously a gymnosperm, the only groups within the gymnosperms to which any relationship at all can be suggested are the Cycadales and Ginkgoales, but even these comparisons must be regarded as distant and tentative. *Beaniopsis* bears some resemblance to the female fruits described by Carruthers under the generic name *Beania* from the Jurassic of Yorkshire. This genus was formerly regarded as a member of the Ginkgoales (Seward 1900 p. 276) but Harris's recent work (1941, pp. 82-97) has shown that it resembles in all important characters the female cone of a typical living Cycad.

The specimens from Onthea, however, exhibit considerable differences from those described under *Beania*. The differences lie in the size of the cone axis, in the shape and size of the sporophylls and seeds in the mode of attachment of the sporophyll stalks to the cone axis and in the mode of attachment of the seeds to the sporophyll. In consideration of these distinguishing features the specimens from Onthea have been placed in a separate genus which has been named *Beaniopsis* in order to indicate that it may possibly have some affinity with *Beania*.

Specimens of this type have not so far been recorded from anywhere in India. Our knowledge of the genus is still necessarily imperfect owing to the difficulty of obtaining complete specimens but it may be that with the discovery of further material *Beaniopsis* may ultimately prove to be, like *Beania*, a rare type of fossil Cycadean fructifications.

*Beaniopsis rajmahalensis* sp. nov.

(Pl. VII, Photos 1-6, Pl. VIII Photos 7-11, Text fig. 1-2)

(Specimens 0/17-0/24)

*Specific Diagnosis*

*Female cones about 1.5 cm. long with a slender longitudinally striated axis less than 1 mm. thick. Sporophylls distant, attached alternately by means of slender stalks 2-3 mm. long and less than 5 mm. wide which usually arise at angles of 60°-70° from the cone axis. The apex of each sporophyll is differentiated into a spoon-like structure bearing two ovoid platyspermic*

seeds each about 1 mm  $\times$  2 mm. The seeds are usually seen to lie with their principal planes in continuation with each other but the orientation of this plane with reference to the median plane of the sporophyll stalk is still difficult to determine. The long axes of the two seeds in a pair diverge from each other at an angle varying from about 60° to 130°. The micropylar ends of almost all the seeds are embedded in the rock matrix and the broad chalazal end only is visible. At this end the principal plane of each seed is distinctly marked by a median longitudinal line.

**Locality**—Ontheri in the Rajmahal Hills. **Collection**—Sahni and party (January 28 1942). **Horizon**—Rajmahal Series (Upper Gondwana).

#### *Cone axis*

The longest fragment has a cone axis about 1.5 cm long but it is quite likely that the original axis was somewhat longer. The rock is quite hard and fractures very irregularly hence all efforts to develop the specimens failed. The axis in the largest specimen (O/21 shown in Photos 1–3) which may be regarded as the type of the species is hardly 1 mm wide. The sporophylls arise from the axis in a spiral manner at rather long intervals. The length of the internode is about 2 mm. Cones with attached sporophylls are preserved in only three specimens (O/21 O/17 O/23) shown in Photos 1 3 4 11. In all other specimens there are well preserved seeds but only fragments of the cone axis are available (Photos 6 7 9).

The axis is longitudinally ridged but only two or three ridges are seen throughout its length. At places a ridge from the axis is seen to be continued for some distance into the stalk of a sporophyll. The sporophyll stalks arise from the axis at angles varying between 60° and 70°. A few stalks seem to arise at smaller angles. These are marked S in Photos 1 and 4. These variations may be attributed to different modes of preservation rather than to any fundamental differences in the original specimens. As a rule we may take it that the sporophyll stalks arise from the axis at angles of about 65°.

#### *Sporophyll stalk*

The stalk of the sporophyll is 2 to 3 mm long and less than  $\frac{1}{2}$  mm wide. It is straight and like the axis has a longitudinally wrinkled surface. These wrinkles are probably due to shrinkage on drying as in the male flowers of a modern *Ginkgo* and do not constitute a structural feature.

The distal end of the stalk enlarges to form a spoon-like structure which may be called the 'head'. This bears two seeds on its upper side but the exact manner of attachment of the seeds is not quite clear. In several fairly

clear cases in which the pair of seeds is seen from the chalazal end the median lines marking the principal planes of the two seeds lie in continuation with each other and in the same plane as the long axis of the sporophyll stalk. This seems to be the usual state of affairs. But there is at least one sporophyll (Photo 2) on which the two seeds seem to lie side by side obliquely somewhat recalling the condition in *Cheirokypis* although there are important differences of detail. The exact mode of attachment of the seeds which is so important a diagnostic character must unfortunately be left undecided until better preserved material comes to hand. As a rule the seeds are preserved on almost all the stalks and have not fallen off. This may indicate a rather young stage of development. Some pairs of seeds however occur detached from their sporophylls.

### Seeds

The seeds are somewhat almond shaped and in preservation they assume peculiar forms which makes their interpretation somewhat difficult at first sight. The normal size of each seed is about 2 mm by 1 mm. The micropylar ends of the two seeds in a pair point in a direction away from each other as shown in Text Figs 1-2. The base of the seed is rounded and cordate, towards the micropylar end the seed becomes elongated and flattened so as to form a small tube like structure giving the entire seed a rather flask like form. Some of the rock matrix is enclosed between the two seeds at the base. By chipping the specimen some of the seed were fractured transversely but no details of the structure of the integument could be made out. The bicarinate integument is a marked feature of the seeds the two carinae being seen as very distinct median longitudinal ridges which are continued from base to apex. Not infrequently however the ridge is replaced by a groove (Photos 7-8) in which the rock matrix is seen wedged in. Some times the groove is so deep that at first sight it looks as if there are four seeds instead of two. If a fractured surface of a seed is examined carefully it is seen that this ridge is only a surface feature probably representing the adhering remains of the integument which elsewhere has been broken off from the smooth surface of the nucule. The superficial nature of this ridge is seen in Photo 10 where a seed marked S has been fractured transversely.

Some of the seeds which have been shed have been flattened out in preservation and the micropylar ends of the two seeds diverge from each other at a wider angle than usual. This character is quite common and almost all those seeds which have fallen are flattened in this manner. On looking at specimen 0/18 (Photos 9-10, Text Fig 1) it seems as if this flattened seed is attached by means of its micropylar end but on a close

examination it becomes quite clear that the two seeds have become flattened out in preservation with their micropylar ends pointing away from each other. The ridge is clearly seen only in one seed in the other it is present only at the base. These two seeds may or may not have belonged to the axis near which they lie. Another well preserved specimen bearing a pair of fallen seeds flattened in the above manner is seen in Photo 6. Here the superficial nature of this ridge is quite evident.

The occurrence of this ridge may however be also explained in another manner. Some of the seeds of *Cycas* (Seward 1977 p 25) are at times flattened and have two prominent angles. Similarly three angled seeds have been found in *Ginkgo biloba* (Seward and Gowan 1900 p 124). It is quite likely that the seeds of *Beaniopsis rajmahalensis* were also two angled and this may explain the occurrence of the ridge.

Sometimes the sporophyll stalks seem to have become twisted through almost 180° so as to lie upside down and the 'head' of the sporophyll is seen worn away in such a manner as to make the chalazal ends of the seeds with the attached sporophyll stalks distinctly visible. This condition is clearly seen in Photo 5 where two sporophyll stalks marked s1 and s2 have become twisted and the chalazal ends of the seeds are clearly visible.

The micropylar ends are embedded in the rock matrix but on examining closely the chinks around the seeds it becomes obvious that the two micropyles point away from each other the longitudinal axes of the seeds diverging at a wide angle which varies from 60° to 150°.

#### COMPARISONS

Of the other fossil remains which appear to offer comparisons with *Beaniopsis* special mention may be made of *Beania*, *Ginkgo*, *Cheurolepis* and *Palussya*.

As far as the present data go the closest resemblance appears to lie with *Beania gracilis* Carruthers. Until recently *Beania* was regarded as an extinct member of the Ginkgoales but it is now in the light of Harris's recent work (1941 p 93) to be regarded definitely as the fructification of one of the true Cycadales. If the resemblances with *Beaniopsis* are proved to be based upon a real affinity then our fossils may also be considered to be the female cone of a cycad although of course they are sufficiently distinct from it to be separated under a new generic name.

In *Beaniopsis rajmahalensis* the length of the cone axis is quite small (about 1.5 cm), the size of the seeds likewise is smaller (usually 2 mm by 1 mm) and the sporophyll stalks arise from the axis at an angle less than a right angle.

On the other hand in *Beania gracilis* the mature cone is considerably larger (as long as 10 cm) the seeds are correspondingly larger (the largest being  $16 \times 13$  mm and the smallest  $7 \times 7$  mm) and the sporophyll stalks arise from the axis at right angles. In *Beaniopsis rajmahalensis* the apex of each sporophyll is differentiated into a spoon-like structure bearing two ovoid platyspermic seeds on its upper side. The micropylar ends of the two seeds in a pair point in a direction away from each other and the principal plane of each seed is distinctly marked by a median longitudinal line. In *Beania gracilis* the head of the sporophyll stalk is usually flattened and bears two oval seeds which are placed parallel to the sporophyll stalks with their micropylar ends facing the cone axis.

If we compare *Beaniopsis* with *Ginkgo* we find that the differences are rather numerous. The female flower of *Ginkgo biloba* consists of an axis bearing two terminal sessile ovules, one on each side of the apex, the base of each ovule being enclosed by a small collar the nature of which has been differently interpreted by different authors. Some abnormal flowers have also been found in which the axis bears several ovules irregularly arranged. The pappules bearing the ovules are greatly inclined to the axis. Such an arrangement however does not help us very much to relate *Beaniopsis* with *Ginkgo*. In the *Ginkgo* flowers the ovules are terminal, the apex pointing outwards; on the other hand in *Beaniopsis* they are placed on the upper side of a spoon-like expansion of the sporophyll.

A comparison of *Beaniopsis* with *Cheirolepis* Schimper and *Palissya* Endlicher does not lead us anywhere. Of these two *Beaniopsis* shows a nearer relation with *Cheirolepis*. *Cheirolepis* (Hurmer 1936 p. 39) has oval cones composed of a number of five-lobed cone scales each bearing two seeds. Each cone scale consists of a bract scale and a deeply lobed ovuliferous scale. The ovules lie nearly parallel to the bract scale with their micropyles facing the cone axis. We have seen that in *Beaniopsis rajmahalensis* there is at least one sporophyll on which the two seeds seem to lie side by side obliquely suggesting a distant similarity with the arrangement in *Cheirolepis*. This does not mean much when we know that such an abnormal position of the seeds in *Beaniopsis* is due to a twist in the sporophyll stalk.

*Palissya* (Hurmer 1936 p. 42) has lax female cones. The cone scales are narrow and lanceolate with a pointed apex. Each cone scale bears 10-12 seeds in two rows and each seed has a characteristic cup-like basal cupule. There is here no evidence of the double nature of cone scales as in *Cheirolepis*. If we try to relate *Beaniopsis* with *Palissya* we are confronted with grave difficulties. Suppose we admit that the cone scale functions in the same

way as the sporophyll stalk and further that the basal cupule in *Palissya* is analogous to the cup shaped expansion of the distal end of the sporophyll stalk of *Beaniopsis*, we cannot account for the considerable difference in the number of seeds and their mode of attachment which is entirely different in the two

On this point, however, a closer resemblance would seem to lie with *Stachyotaxus elegans* Nathorst (Nathorst 1908 p 11, Hirmer 1936 p 42, Seward, 1919, p 411) in which the sporophylls arise approximately at right angles from the axis, each sporophyll consisting of a short and thick stalk expanded distally into a scale like outgrowth with a pointed apex and bearing two seeds, each of which is placed in a cupule. But if we were to go into other details, we would find that here also the possibility of any close affinity totally fails

In view of all these points, the real affinities of this extraordinary genus must be left undecided till some more material is available

#### SUMMARY

The interesting forms described in this paper under the new generic name *Beaniopsis* were found at Onithea in the Rajmahal Hills. Most of the specimens show well preserved female cones with distant sporophylls attached alternately to the cone axis by means of slender stalks. The stalk of the sporophyll is straight and like the cone axis its surface is longitudinally wrinkled. The distal end of each stalk is spoon shaped and bears two seeds on its upper surface. The exact mode of attachment of the seeds is not quite clear and must remain unsettled till better preserved material is found. Usually the seeds are placed in such a manner that the median lines marking the principal planes of the two seeds lie in continuation with each other and in the same plane as the long axis of the sporophyll stalk. The seeds are somewhat almond shaped with the base rounded and cordate, and the micropylar end is elongated and flattened. The micropylar ends of the seeds in a pair diverge from each other at a wide angle.

The genus is evidently a gymnosperm and the only groups to which a relationship can be suggested, with the present data in hand, are the Cycadales and less probably the Ginkgoales.

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## EXPLANATION OF PLATES

All photographs are untouched.

The figured specimens are preserved in the Department of Botany and Zoology Lucknow University.

## PLATE VII

*Beaniopsis rajmahalensis* gen. et sp. nov. (Figs 1-6)

- Photo 1 Two female cones lying near each other. Each bears a number of alternately attached sporophylls. The sporophyll stalks marked S are seen to arise from the cone axis at a smaller angle than usual. 0/21 2.
- Photo 2 The same specimen enlarged to show the nature of cone axis and of the sporophyll stalk. On one sporophyll (s) the two seeds seem to lie side by side obliquely. 0/21 4.
- Photo 3 Part of a female cone. The cone axis shows longitudinal striations distinctly at one or two places. 0/21 2.
- Photo 4 A well preserved female cone bearing alternately attached sporophylls. The sporophyll stalk marked S is seen to arise from the cone axis at a smaller angle than usual. 0/17 2.
- Photo 5 The same specimen enlarged. The sporophyll stalks marked s1 and s2 have been twisted the head of the sporophyll is seen worn away and the chalazal ends of the seeds with the attached sporophyll stalks are clearly visible. 0/17 Ca 34.
- Photo 6 A pair of seeds flattened out and exposed by their chalazal ends. The micropylar ends of the two seeds seem to diverge from each other at a rather wide angle. 0/17 x 13.

## PLATE VIII

*Beamopsis rajmahalsensis* gen et sp nov (Photos 7-11)

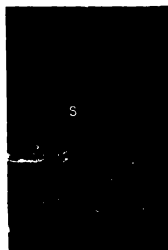
- Photo 7 Two pairs of seeds lying side by side. Each seed shows a distinct median longitudinal groove continued from its base to apex. In the upper part of this photograph fragments of cone axis are seen. 0/19  $\times$  2
- Photo 8 The same specimen enlarged. Towards the top left of this photograph the round and cordate base of a seed is seen rather well preserved.  $\times$  Ca 6
- Photo 9 Fragments of cone axis with a few attached sporophylls. The position of a pair of well preserved seeds lying near an axis is indicated by means of an arrow. 0/18  $\times$  2
- Photo 10 A portion of the same specimen enlarged to show the pair of seeds referred to above. Each seed is flattened and shows a distinct median longitudinal ridge continued from its base to apex. The micropylar end of the seed is flattened out and the base is rounded and cordate. Towards the top left of this photograph a seed marked S is fractured transversely and reveals the superficial nature of the ridge.  $\times$  Ca 6
- Photo 11 Fragment of a female cone. 0/23  $\times$  2



13



14



15



16



17 ( $\times 2$ )



18 (ex 34)

Fig. 1—(P) 110p c 11 111 dens



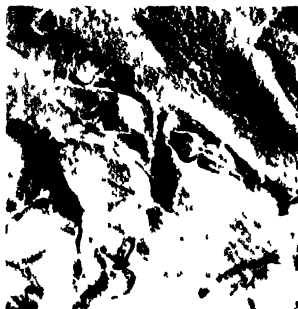
8 a



7 ( $\times 2$ )



11 ( $\times$ )



10 ( a G)

FIGS 7-11 *Beaopsis rajalensis*

# **ONTHEANTHUS POLYANDRA GEN. ET SP. NOV., A NEW TYPE OF FOSSIL GYMNOSPERM MALE FRUCTIFICATIONS FROM THE RAJMAHAL HILLS**

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(With four Plates and four Text Figures)

CONTENTS	PAGE
INTRODUCTION	105
DESCRIPTION	107
Generic diagnosis	107
Specific diagnosis	108
General structure of the flower	109
Receptacle, peduncle, mode of attachment of flower	110
Number of sporophylls in the flower	110
Form and structure of the microsporophylls	110
The microsynangia	112
DISCUSSION	112
Nature of the parent plant	Reconstruction
Affinities	113
SUMMARY	115
BIBLIOGRAPHY	116
EXPLANATION OF PLATES	117

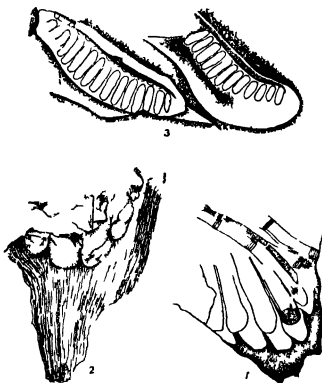
## INTRODUCTION

THE fossils here described were collected on 28th January 1942 at Onthea, a village in the province of Behar by a party of research students of Lucknow University led by Professor Birbil Sahni, F.R.S. The author then a research student was also a member of the party. The locality of Onthea was previously known to earlier investigators like Oldham, Morris, and Faistmantel. The exposure of the plant-bearing strata is not far from the village (Ganju, 1946, Text Fig. 1). Almost all the fossils were collected from beds *in situ*, and, therefore, there is no doubt that they belong to the Rajmahal Series. The different forms were found on breaking hard, fine grained, siliceous rocks. A few of the 'flowers' were found by Dr. R. V. Sitholey while he was breaking some pieces of the big slab (Ganju, 1946, Introduction). I am

extremely grateful to Dr. R. V. Sitholey who has been kind to hand over these specimens to me for description.

The entire work was carried out under the close supervision of Professor Sahní. The figured specimens are kept in the museum of the Department of Botany and Geology Lucknow University.

It is matter of very great pleasure to express my indebtedness to Professor Sahní for the ungrudging help and valuable guidance he has been constantly giving me. It is needless to say that without his suggestions it would have been well nigh impossible for me to bring up this work to its



*Otilantia polyantra* gen. et sp. nov.

TEXT FIG. 1. A number of microsporophylls viewed from their adaxial surface and lying on the inner side of a portion of the perianth. Each sporophyll is divided into two parts: (i) a very short sterile basal portion, broadly rounded at its lower end and continued distally into (ii) a long and narrow very gradually tapering scale. 0/30  $\times$  1½.

TEXT FIG. 2. Basal portions of four or five sporophylls lying on the inner surface of the perianth. 0/45  $\times$  2½.

TEXT FIG. 3. Lateral view of two uppermost sporophylls of the specimen shown in Photo 10. These sporophylls have become folded longitudinally along the middle line so that the two rows of stamens make an acute angle with each other. 0/72  $\times$  Ca. 6½.



within a cup like perianth. Each sporophyll bears two contiguous rows of crowded transversely elongated synangia.

Under this genus are included some gymnosperm fructifications of a very interesting new type which were found only at Onthar. The only species of this genus *Onthecanthus polyandra* sp. nov. is represented by at least fifteen specimens. All the specimens are incomplete but a provisional reconstruction of the numerous fragments suggests that here we have an altogether new type of male fructifications probably belonging to one of the Bennettitales. A large number of elongated microsporophylls rounded at the base and gradually narrowing towards the distal parts are attached in a crowded manner all round a thick elongated receptacle. The sporophylls bear numerous narrowly oblong sporangium like organs placed transversely in two contiguous rows. Some of these sporangium like bodies show definite signs of partition into chambers. They must therefore be regarded as microsynangia although no spores have yet been found in them. The entire receptacle with its mass of sporophylls is enclosed in single cup like perianth. The internal surface of this perianth and the surface of the receptacle are peculiarly wrinkled.

No definite views can be expressed regarding the affinities of this fossil. As will be shown later this fructification seems to be a male flower of some peculiar type of Bennettitales resembling in some respects the genus *Cycadeoidea*.

*Onthecanthus polyandra* sp. nov.

(Pl. IX Photos 1-4 Pl. X Photos 5-7 Pl. XI Photo 8-11)

Pl. XII Photos 12-14 Text Figs 1-4)

(Specimens 0/30 0/37 0/39 0/46 0/71 0/75 0/80 0/103)

*Specific diagnosis*

Flower pedunculated at least 7 cm long. Peduncle about 1 cm thick. Perianth deeply funnel shaped about 5.5 cm long and 3.3 cm in the broadest part with the inner surface longitudinally wrinkled. Receptacle at least 3.5 cm long 2 mm in its broadest middle portion gradually narrowing towards the base and apex. The surface of the receptacle is longitudinally corrugated in the same manner as the inner surface of the perianth. Androeceum exerted. Sporophylls very numerous at least 120 but probably many more in each flower attached in a densely crowded manner all round the receptacle throughout its length and overlapping each other. Each sporophyll is attached by a thick and broadly rounded sterile base about 3 mm  $\times$  1 mm. This is continued distally into a long and narrow very gradually tapering scale about 2.5 cm long and

about 3 mm to 1.5 mm broad which bears on its adaxial surface two closely set rows of narrowly oblong synangia. The two rows of synangia are contiguous with each other being separated only by a narrow median line. They cover the whole upper surface of the scale (excluding only a very narrow marginal strip of the sterile part of the sporophyll on either side). The synangia in each row are also contiguous: those in the broad basal part of the scale (next to the sterile base) are about 1.4 mm  $\times$  .5 mm; those placed more distally about 7 mm  $\times$  .3 mm. Some of the synangia show clear signs of being divided as in *Cycadeoidea* into a number of chambers which lie on the two sides of median partition. Spores not seen.

**Locality.** Onthera (about four miles due South of Maharajpur railway station) in the Rajmahal Hills, Bihar. Collection: Sahoo and party (January 28, 1942). **Horizon.** Rajmahal Series (Upper Gondwana).

#### *General Structure of the Flower*

The above diagnosis is based upon a reconstruction of about fifteen specimens, none of which is complete. The most complete flower which is to be regarded as the type specimen is shown in Photo 1. This represents a longitudinally fractured flower; the other half of which was broken while splitting the rock. The portion preserved measures about 6.5 cm long and 3.3 cm wide. It shows an outer continuous cup-like envelope (c) serving as a perianth. The distal part of the perianth is not preserved and therefore it is impossible to say whether it was a continuous structure or lobed as in most Bennettitalean flowers.

That the fracture through this flower has passed somewhat tangentially is evident from the fact that we see a number of sporophylls (about eight or nine) placed longitudinally even in the middle of the specimen (Photo 1). These must have been attached on the side of the receptacle facing away from the receiver for they all present their adaxial (that is, synangium-bearing) surface. The sporophylls attached along the right and left sides of the section are only seen cut through their sterile swollen bases, which thus appear like two longitudinal series of lobes.

It is not easy to estimate the thickness of the receptacle in a tangential section but it appears to have been at least 2 cm thick and this estimate agrees more or less with what we find in Photo 4.

The entire hollow of the perianth must have been filled up by the receptacle and its attached microsporophylls. The distal parts of the sporophylls must have expanded out from the perianth like the exerted stamens of a modern flower. The internal surface of the perianth and the surface of receptacle are longitudinally wrinkled throughout (Photos 1-4).

*Receptacle Peduncle Mode of Attachment of Flower*

In Photo 4 we see two flowers lying almost parallel with each other, possibly attached to one and the same axis which however is not preserved. In each of these the flowers longitudinally wrinkled lower half of the receptacle is seen gradually narrowing downwards into a thin cylindrical peduncle especially well seen in the right hand flower. Attached on the upper part of each receptacle we see (especially in the left hand flower) the swollen basal parts of a few sporophylls which are seen continued distally into their narrow fertile portions.

*Number of Sporophylls in the Flower*

The number of microsporophylls in each flower must have been very large indeed to judge by their densely crowded arrangement. Some idea of their number may be gained by examining Photos 1 2 4 5. In the middle region of Photo 1 we see as already stated about eight or nine sporophylls placed in a transverse row roughly parallel to each other. The number of sporophylls met with in passing along a vertical section (as represented by the lobes at the right side of the photograph) is about eight. Assuming that the other half of the flower also contained the same number, if not more we may take it that on an average there were in all about 128 to 144 such scales in each flower.

Roughly the same sort of estimate is arrived at by examining Photo 2 which also represents a tangential section through a flower although here the perianth is only preserved on one side (p). Here too we find that in passing transversely across this photograph eight or nine sporophylls are met with placed parallel to each other as in Photo 1. In the lower part of the photograph the swollen bases of a few of these sporophylls are seen. The number of sporophylls traversed in passing from below upwards is not clear but if we compare Photo 10 it is evident that this number must be not less than seven.

Photo 4 illustrates the considerable length which the receptacle must have attained and also shows (see left hand flower) that it was fertile even in its narrow distal portion which here is clearly seen bearing a vertical series of thick sterile lobes tapering upwards into their narrow fertile scales.

*Form and Structure of the Microsporophylls*

The microsporophylls reach a length of about 2.5 cm. Each sporophyll is divided into two parts (i) a very short sterile basal portion about 3 mm x 1 mm broadly rounded at its lower end and concave along its upper margin. This continued distally into (ii) a long and narrow very

gradually tapering scale, about 2.5 cm long and 3 mm to 1.5 mm broad. It is likely that the scales reached a considerably greater length and projected well beyond the perianth. These bear on their adaxial surface two closely set rows of narrowly oblong synangia. It is by the short sterile basal portion that the sporophyll is attached to the receptacle. The longitudinal section in Photo 1 shows clearly on the right-hand side the attachment of seven or eight sporophylls of which only their basal portion is visible. The same kind of structure less clearly preserved, is seen in the left-hand side of the photograph. In Photo 5 (Text-Fig 1) we see four or five sporophylls viewed from their inner (adaxial) surface and lying on the inner side of a portion of the perianth. The same specimen is shown slightly enlarged in Photo 6 and still further enlarged, to show the details in Photos 7, 8 and 12. In several of the sporophylls the sterile marginal strips are seen as thickened ridges extending along the two sides of the sporophyll, especially in their basal portions. Covering a portion of the third sporophyll from the right are detached strips of the abaxial sterile portions of one or two sporophylls which lay above them (that is, in a position internal to them with respect to the receptacle) and were broken off with the counterpart.

The basal parts of the scales are seen to overlap one another. Those which pass along the plane of section are seen complete. The microsporophylls are closely packed leaving no space between them.

In the specimen shown in Photo 1 it looks as if the sporophylls are attached to the inner surface of the perianth but this appearance must be deceptive. This flower has accidentally been fractured tangentially, and the portion of the perianth on which the sporophylls appear to be attached is seen from the inner surface to which the sporophylls have remained adhering. A somewhat similar specimen is seen in Photo 2 which has already been described above.

In Photo 10 we see a lateral view of a row of about seven sporophylls attached to the receptacle (*r*) and viewed from their abaxial sides. Of these the three uppermost microsporophylls are further enlarged in Photo 11 to show clearly the form and arrangement of the synangia. The synangia have been brought into view by the removal of the abaxial sterile tissue of some of the sporophylls except for one narrow strip marked *s* (see also Text-Figs 3, 4). Specimen 0/33 (Photo 3) which in its general features is comparable with Photo 5, shows relatively narrower and longer sporophylls, lying on the inner surface of a portion of the perianth. Specimen 0/45 (Photo 9, Text-Fig 2) shows the basal portions of four or five sporophylls lying on the inner surface of the perianth.

*The Microsynangia*

The microsynangia are continued in numerous narrowly oblong sac-like organs arranged transversely in two dense rows one row on either side of a median line. They lie quite flat usually making an angle of  $90^\circ$  with this line (Photo 12). Some sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other (Photos 7-11 Text Figs 3-4). As a rule however the adaxial surface of the sporophyll is flat so that all the synangia lie in one plane. Text fig 3 has been drawn on an enlarged photograph of the specimen shown in Photo 10. These are the two uppermost sporophylls of the row. Here a strip of the adjacent sterile tissue is clearly seen to bear on its upper (adaxial side) the two rows of synangia which make an acute angle with each other. Text Fig 4 shows a camera lucida sketch of the uppermost sporophyll still further enlarged.

Each synangium is about 7-1.5 mm long and 3 to 5 mm broad so that in a longitudinal distance of 1 mm two or three of them are traversed. In specimens 0/71 0/30 and 0/31 (Photos 2, 12 and 13 respectively) some of the synangia when carefully examined with the help of a pocket lens from all directions seem to be divided into a number of chambers along a faint median line somewhat like the synangia on the micro-sporophylls of *Cycadeoidea*. Some other specimens however (Photo 11) only reveal the presence of a number of small rounded raised areas probably marking the position of the individual sacs of the synangia. Attempts to obtain spores have however completely failed. Portions of synangia were broken off for maceration. Both concentrated nitric and hydrochloric acid were tried but the rock did not disintegrate. A careful examination of the exposed surface of the synangia under the high power of a microscope also did not reveal the presence of any spores.

**DISCUSSION***Nature of the Parent Plant Reconstruction*

It is difficult to say anything definite about the nature of the parent plant and the manner in which these flowers were borne on it. Fortunately, however a specimen (No 0/73) has been found which enables us to gain some idea of the kind of stem on which the flowers were attached. This specimen shown in Photo 14 shows a branched axis about 10 cm long and 8 mm broad wrinkled in just the same fashion as the inner surface of the perianth and the exposed surface of the receptacle of *Ontheanthus polyandra* sp. nov. Towards the left side (at f) is preserved a fragment of a flower of

the same species, attached to a short stalk and bearing a number of well preserved microsyngangia of the characteristic type already described. The angle at which this stalked flower lies with respect to the long axis just mentioned is such as to leave no doubt that the stalk was continued downwards and was fixed to the main axis near the point marked x. Higher up on the same axis another branch is seen to arise towards the right. This branch is again wrinkled in the same manner as the main axis and must also have borne a flower of *O. polyandra* but of this flower there is no direct evidence, because the branch is broken off distally.

In another specimen (No. 0/42) shown in Photo. 4 which has already been described above, two flowers are placed nearly parallel to each other. These may have been attached below to one and the same branch bifurcating near the apex, each branch ending in a flower.

#### *Affinities*

The flower described here does not resemble even in broad features, any genus hitherto known, hence it is extremely difficult to explain its affinities. It is an entirely new type of fructification and most of its features are so unique as to make its study very interesting. For the same reason its interpretation is a matter of some difficulty with the result that the morphology of the flower is in some respects still rather obscure.

Although a perianth is present a reference to angiosperms is obviously out of the question. Knowing that these flowers come from beds of Jurassic age, the only groups which can come into consideration are the gymnospermous phyla Coniferales, Cycadales and Bennettitales. A comparison with the Coniferales and Cycadales is ruled out because of the presence of a definite cup-shaped perianth and the structure of the sporophylls. With the Bennettitales, however, these flowers do show at least some broad features in common which suggest that it is a male fructification of some unusual type either belonging to or related to this diversified phylum which is so strongly represented in the Rajmahal flora. Let us consider these points in some detail.

Firstly, the general organisation of *Ontheanthus polyandra* is just like that of a Bennettitalean flower. There are numerous sporophylls attached to a thick receptacle which is enclosed by a perianth.

Secondly, the structure of the sporophylls is strongly suggestive of a Bennettitalean affinity, the presence of syngangia divided into chambers as in *Cycadeoidea*, and their biserial arrangement, being important points specially worthy of consideration.

As we shall see however, there are also equally important points of difference

As regards the feature in which *Ontheanthus* differs from the *Bennettitales* there are three (i) The mode of attachment of the microsporophylls, which instead of being placed in a single whorl are disposed spirally in a densely crowded manner on a thick elongated receptacle (ii) The absence of a basal cup-like portion of the andræcium such as we find in *Williamsonia* *Cycadeoidea* and other *Bennettitales* (iii) The presence of a cup-like perianth

The nature of the associated vegetative remains *e.g.* of leaves, also sometimes help in indicating the affinities of a new type of flower, but one must not attach too much importance to mere associations. The only leaves found in close association with these flowers are those of the *Ptilophyllum acutifolium* type. We know that the leaves known as *Ptilophyllum cf. cutchense* were almost certainly the foliage of *Williamsonia Sewardiana* but the nature of the fructifications borne by the parent plants of the other Indian *Ptilophyllums* are still unknown. It is not impossible that one of the component forms of the aggregate "species" *P. acutifolium* may have been the foliage of the plant which bore the flowers here described as *Ontheanthus polyandra*. It is true that the branched vegetative axis (Photo 14) which bore the flower of *Ontheanthus* is wrinkled throughout and shows no trace of a rhomboid leaf scar such as a *Ptilophyllum* might be expected to leave on a stem. But it is not inconceivable that the flowering shoots of *Ontheanthus* bore only small scale-like leaves while the mature vegetative stem carried foliage leaves of the *Ptilophyllum* type. At the same time it must be confessed that all this is mere speculation. the foliage of *Ontheanthus* is still unknown.

No evidence of the female parts of *Ontheanthus* has been yet found, although of the male flowers as many as fifteen specimens have been found. It is not likely that any ovulate organs were attached to the receptacle of the species here described as *O. polyandra*, because the receptacle bearing the microsporophylls is quite thick and fills the entire hollow of the perianth, leaving no room for the attachment of female organs. But some *Bennettitales* were certainly unisexual and others hermaphrodite. It is, therefore, quite probable that in this new form we have an unisexual type of flower and that the female flower of this type is still to be discovered. A further search at *Onthea* may reveal its presence there.

Considering all these facts, we evidently have in *Ontheanthus* an entirely new and a distinct genus of very great interest which, while showing

Bennettitalean affinities at the same time exhibits peculiar features of its own. In the form and arrangement of sporophylls and in the structure of synangia this new genus may be compared to the genus *Cycadeoidea* in some of its broad features. But in the densely crowded spiral mode of attachment of the sporophylls which moreover are placed all over the extensive surface of a thick and elongated receptacle it is so far away a unique type among the Bennettitales. The cup-like perianth is also a unique feature.

The fossils described here have been given the new name *Ontheanthus polyandra* gen. et sp. nov. This name has been chosen in order to denote a male flower from *Onthea* containing numerous micro sporophylls. This locality was known to Oldham as early as in 1863 (see *Oldham and Morris 1863 Preface p. xv*) and to Feistmantel in 1877 (*Feistmantel 1877 p. 6*) but it is only in recent years that extensive search at this locality has begun to reveal a number of interesting new types of plants. Among these *Beaniopsis* (Ganju 1946 a) *Ontheanthus* and *Ontheoistrobus* (Ganju 1946 b) are three of the most important forms (Ganju 1944 pp. 76-77).

#### SUMMARY

The new type of male fructification described in this paper under the name *Ontheanthus polyandra* is fairly common at *Onthea*. The flower consists of a short peduncle and a deeply funnel-shaped perianth. The receptacle is about 2 cm. thick and must have been at least 3.5 cm. long. The exterior of the receptacle and the inner surface of the perianth are wrinkled. The sporophylls are numerous and crowded throughout the length of the receptacle. Each sporophyll consists of a heart-shaped broadly rounded basal portion which passes distally into a long and a narrow caudex tapering very gradually and projecting beyond the perianth. The micro-sporophylls bear on their adaxial surface two closely set rows of narrowly oblong synangia arranged transversely, one row on either side of a median line. The synangia lie quite flat usually making an angle of 90° with this line but some sporophylls have become folded longitudinally in a peculiar manner with the result that the two rows of synangia make an acute angle with each other.

It is rather difficult to say anything definite regarding the nature of the parent plant and the manner in which these flowers were borne on it. It is equally difficult to be certain about the real affinities of such an unusual type of fructification. All that can be said at present after taking into consideration the broad features only, is that our flower is a male fructification.

probably of one of the Bennettitales. In this group the only plausible comparison in respect to the form and the arrangement of sporophylls is with the genus *Cycadeoidea*.

This find of an interesting new type of fructification is a notable addition to other interesting forms already known from the Jurassic beds of the Rajmahal Hills. The Bennettitales were at the height of their development during the Jurassic period and *Ontheanthus polyantra* comes from beds definitely known to be of Jurassic age.

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EXPLANATION OF PLATES

All photographs are untouched. They are of natural size except where the magnification is given.

The figured specimens are preserved in the Department of Botany and Geology, Lucknow University.

PLATE IX

*Ontheanthus polyandra* gen et sp nov (Photos 1-4)

- Photo 1 A longitudinally fractured flower. The outer continuous cup-like envelope (*c*) is the perianth. On the right hand side the attachment of basal portions of seven or eight sporophylls is visible. The same kind of structure, less clearly preserved, is seen in the left hand side of the photograph.  $0/71 \times Ca\ 1\frac{1}{2}$
- Photo 2 Portion of a flower fractured tangentially. The perianth (*p*) is only preserved on one side. A number of microsporophylls parallel to one another can be clearly seen. This flower lies near the one shown in Photo 1.  $0/71 \times Ca\ 1\frac{1}{2}$
- Photo 3 The sporophylls in this flower are relatively narrower and longer.  $0/33 \times 1\frac{1}{2}$
- Photo 4 Two flowers lying almost parallel with each other. A portion of the peduncle is especially well seen in the right hand flower. The longitudinal wrinkles on the surface of the receptacle are clearly visible.  $0/42$

PLATE X

*Ontheanthus polyandra* gen et sp nov (Photos 5-7)

- Photo 5 A number of closely packed microsporophylls viewed from their adaxial surface and lying on the inner side of a portion of the perianth. The basal parts of the sporophylls are seen to overlap one another.  $0/30$
- Photos 6-7 The same specimen enlarged to show the structure of microsporophylls. The sterile marginal strips are clearly seen as thickened ridges extending along the two sides of the sporophyll. Some sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other. Photo 6  $\times 2$ , Photo 7  $\times Ca\ 3$

PLATE XI

*Ontheanthus polyandra* gen et sp nov (Photos 8-11)

- Photo 8 Specimen shown in Photo 5 enlarged to show the broadly rounded short sterile basal portion of the sporophyll. The microsynangia are contained in narrowly oblong sac-like organs arranged transversely in two dense rows, one row on either side of a median line.  $\times 4$
- Photo 9 The basal portions of four or five sporophylls lying on the inner surface of the perianth.  $0/45 \times 1\frac{1}{2}$
- Photo 10 Lateral view of a row of about seven microsporophylls attached to the receptacle (*r*) viewed from their abaxial sides.  $0/72 \times Ca\ 2$
- Photo 11 The same specimen enlarged to show the three uppermost microsporophylls. The synangia have been brought into view by the removal of the abaxial sterile tissue except for one narrow strip marked *s*. The sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other.  $\times 4$

## PLATE XII

*Ontheanthus polyandra* gen et sp nov (Photos 12-14)

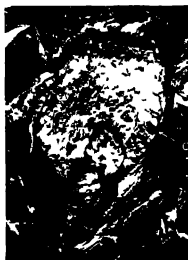
- Photo 12 Part of the specimen shown in Photo 5 enlarged. On some microsporophylls the microsynangia lie quite flat usually making an angle of  $90^\circ$  with the median line  $\times Ca$  5
- Photo 13 Two sporophylls lying side by side. Some of the microsynangia show rather clearly the presence of a faint median line 0/31  $\times 4\frac{1}{2}$
- Photo 14 A branched axis which bore the flowers. At *f* is preserved a fragment of a flower attached to a short stalk which was fixed to the axis near *x*. Another branch is seen to arise at *r* 0/73 9/10 Nat size



1



(c. 14)



1



2 (c. 11)

Fig. 1-4. *Ostrea p. laticosta*

*P. N. Ganje*

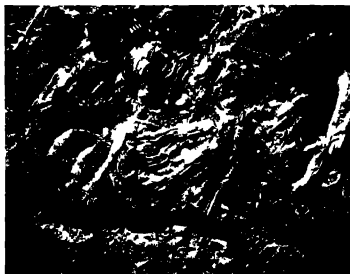
*P. Int. Acad. S. I. I. V. P. I. V.*



5



7



9

*I c 5 O H s / 1 1*

*I Δ C*

*I o I t I c I Se I I \ \ I Pl \ I*





FIGS 12-14 *O. t. ca. l. s. poly. dr.*

# **ONTHEOSTROBUS SESSILIS GEN ET SP NOV , A NEW TYPE OF SEED-BEARING GYMNOSPERM FRUCTIFICATIONS FROM THE JURASSIC OF ONTHEA IN THE RAJMAHAL HILLS**

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(Communicated by Prof B Sahni FRS)

(With two Plates)

CONTENTS	PAGE
INTRODUCTION	119
DESCRIPTION	120
Generic diagnosis	120
Specific diagnosis	120
The receptacle mode of attachment of seeds	121
The structure of the seeds	122
AFFINITIES	123
SUMMARY	123
BIBLIOGRAPHY	124
EXPLANATION OF PLATES	125

## INTRODUCTION

THE interesting material which forms the subject matter of this paper was found at Onthea on 28th January 1942 when also the forms described earlier (Ganju 1944 pp 76 77 1946 1946 a 1946 b) were collected Onthea has now turned out to be a very interesting and useful place for the study of fossil plants The forms described in all these four papers were found in a single day's field work One can easily imagine the wealth of material that the fossiliferous outcrop at Onthea (Ganju 1946 Text Fig 1) may be expected to yield on a detailed study

Some of the forms here described were seen on the exposed weathered surface of the specimens while some were discovered only on breaking the familiar kind of hard grey siliceous rocks so commonly found at Onthea Dr R V Sitholey found a few specimens while breaking the big slab a detailed reference to which has been given in an introduction to the first paper (1946) I am very thankful to Dr Sitholey for kindly giving me these specimens for description

The work has been carried out, as usual under the guidance of Professor Birbal Sahni, F.R.S., to whom I owe sincere thanks for helping me to plod through many of the difficulties that so often confront one in a new field of research. I have to thank Mr. R. N. Laxmanpal for correcting the proofs of this paper.

An extremely interesting new type of seed-bearing gymnosperm fructification is described in this paper. It is not possible yet to say anything definite about the real affinities of this form but at first sight a distant comparison with the Bennettitales is suggested. The absence of interseminal scales, however, remains an obstacle in including this genus within that phylum. For the present, therefore, the systematic position of this type of fructification must remain unsettled.

#### DESCRIPTION

##### *Ontheostrobus* gen. nov.

##### *Generic diagnosis*

*Megastrobilus* consisting of an axis bearing numerous crowded sessile seeds on all sides. Seed orthotropous seated in a shallow saucer shaped cupule sessile on the axis with a central point marking the vascular supply. Chalazal end of detached seed shows a large elliptical scar of attachment, micropylar end not preserved.

This genus too is very well represented at *Onthea* and is based upon a number of well preserved though incomplete specimens. The "flower" consists of a much elongated axis which functions as a female receptacle bearing numerous crowded orthotropous seeds all round it. There are no megasporophylls in the ordinary sense but the seeds are borne directly or almost directly upon the receptacle, with only a small intervening basal cup-like structure which itself is sessile on the axis. With the meagre data at hand it is impossible to say anything about the real affinities of the plant. At first sight a distant comparison with the Bennettitales may suggest itself, but there is no indication of any interseminal scales, nor has a perianth yet been discovered. For the present the systematic position of this extraordinary type of fructification must remain an open question.

##### *Ontheostrobus sessilis* sp. nov.

(Pl. XIII, Photos 1-5; Pl. XIV, Photos 6-9)

(Specimens 0/76-0/79, 0/81-0/90, 0/102, Also the big slab from *Onthea*)

##### *Specific diagnosis*

A much elongated receptacle bearing numerous sessile closely crowded seeds (broadest diam. about 6 mm.) all round through its length. Receptacle

about 3.5 cm long (actual base and apex not preserved) about 1.6 cm  $\times$  1 cm in diameter at the base (? flattened by pressure) above which it rapidly narrows down to about 7 mm  $\times$  4 mm diam and then tapers very gradually from below upwards to about 3 mm diam at the top. The seed is placed in a shallow cupule which itself is seated on a cushion; the cushion in turn is sessile on the axis. Where the seeds have fallen off a number of elliptical areas are left on the receptacle—these are the exposed surfaces of the cushions which are themselves slightly hollowed out and show in the centre a small pit marking the vascular supply of the seed. The micropylar end of the seeds is never preserved. The detached seeds have a large elliptical scar (about 2 mm  $\times$  3 mm) at the chalazal end evidently showing the area of attachment with the cupule.

**Locality** Onthea in the Rajmahal Hills. **Collection** Srinivas and party (28th January 1942). **Horizon** Rajmahal Series (Upper Gondwana).

*The receptacle—mode of attachment of seeds*

Of about ten more or less complete specimens collected at Onthea five show well preserved receptacles with some of the seeds still attached round them (Photos 3, 6, 8, 9) while the remaining specimens only show groups of seeds detached from the axis but still packed into closely crowded groups and exposed by their chalazal ends (Photos 5, 7).

Specimens No. 0/77 (Photos 1–3) when split revealed the presence of a large number of seeds attached to the long tapering receptacle. The piece which came off first is shown in Photo 1 and the receptacle is shown in Photo 3. A similar number of seeds was expected to be attached on the other side of the receptacle and on careful splitting another piece came off which is shown in Photo 2. Thus all the seeds represented in the Photos 1–2 were attached round the receptacle shown in Photo 3.

It is very difficult to form an idea of the entire length of the receptacle because the actual base and apex are not preserved but the portion exposed measures about 3.5 cm long. At the base it is 1.6 cm  $\times$  1 cm. In passing from below upwards at first it rapidly narrows to about 7 mm  $\times$  4 mm and then tapers very gradually towards the apex (Photo 3). This is the most complete specimen and it is regarded as the holotype. Here the size of the seeds and of the scars (measuring about 3 mm  $\times$  2 mm at the base and about 2 mm  $\times$  1.5 mm at the apex) left by them on the receptacle decreases towards the narrow end of the receptacle hence the thicker end is taken as the proximal part and the thinner end as the distal. About 75 more or less complete seeds may be counted in this specimen and these were attached all round the receptacle in a length of about 3.5 cm. Thus the

total number of seeds on the complete receptacle must have been very considerable.

Axes of a similar type is preserved in some other specimens, e.g., 0/76 (Photo 9) 0/78 (Photo 8) 0/79 0/81 (Photo 6) etc. but none is so complete as the one just described.

A few seeds still attached to the receptacle are well seen in specimen 0/81 shown in Photo 6. On carefully observing this photograph it appears as if the seeds were enclosed in a shallow membranous saucer-like or cup-like structure. But the preservation is not good enough to show whether this membranous structure was a cupule-like organ distinct from the seed or whether it only represents the persistent basal part of the integument of the seed, the rest of which is not preserved.

Receptacles from which all seeds have fallen off are also present. These usually bear a number of characteristic circular or elliptical scars left by the seeds. One of these receptacles appears in Photo 4. The elliptical scar is a raised cushion slightly hollowed out on its exposed surface, in which the seed is seated. In the centre of this depression a small pit can be made out (marked *p* in this photograph). These pits probably mark the vascular supply of the seeds. Photo 5 shows a group of seeds which were once attached on this particular receptacle but which came off as a single piece while splitting the specimen. The seeds are all exposed by their chalazal ends, and show the characteristic elliptical scar of attachment, in the centre of which a distinct pit is sometimes seen marking the vascular supply.

Photo 8 shows another specimen consisting of numerous seeds crowded together in their original grouping. In the upper part of the figure a small portion of the receptacle (*r*), is still left intact. The side of the receptacle facing the reader shows a number of scars left by the seeds which were broken off with the counterpart. Where the receptacle is not preserved, the seeds are exposed by their chalazal ends and some of these show clearly an elliptical scar, representing the surface of attachment with the receptacle.

### *The Structure of the Seeds*

We have seen that the seeds are closely packed and no interseminal scales are present. The detached seeds too have an elliptical scar at the chalazal end (Photo 7) corresponding to the elliptical scar on the receptacle and evidently showing the area of attachment. This area of attachment measures about  $2 \times 3$  mm, above this the seed gradually widens out to about 6 mm diam. The shape and size of the entire seeds is difficult to tell because the micropylar end is never preserved.

# AFFINITIES

With only this much information in hand it is impossible to say anything definite regarding the affinities of this form. These features are not presented by any known gymnosperm fructifications. With the Cycadales no comparison is possible because the organ which bears the seeds cannot by any stretch of imagination be regarded as a sporophyll. It is evidently homologous with a floral axis. With the mention of a floral axis the Bennettitales come to mind but here again there are difficulties because there is no trace anywhere of interseminal scales. If subsequently a perianth is discovered at the base of the axis (which in our material is incomplete) a comparison with the Bennettitales may come within the range of possibility, but even then the absence of interseminal scales will remain an obstacle to our including this genus within the Bennettitales unless we are prepared to extend the present definition of that phylum. There is only one consideration which may still bring this genus within the fold of the Bennettitales and that is the perhaps rather remote possibility that in the younger stage of development interseminal scales of a delicate character were present, but that with the growth of the seeds they became crushed out of recognition. This again suggests that *Onthea* is a locality deserving of a closer attention by palaeobotanists than it has so far received. It is possible that with further search younger stages and more complete specimens of *Ontheostrobus* may be discovered.

From the above consideration alone the extraordinary interest of this genus is self-evident.

It is possible that further research especially when more material is collected may prove some sort of relationship between *Ontheostrobus sessilis* and *Rajmahalia paradoxa* Sahní and Rao, which is regarded by its authors (1935, p. 712) 'as an inverted funnel-like disc (possibly part of a deciduous andræcium) fallen from the top of a Bennettitalean receptacle and bearing on its inner surface the impress of seeds and interseminal scales once pressed against it, but now no longer preserved'.

In view of the sessile nature of the seeds the fossils described here are given the specific name *Ontheostrobus sessilis* sp. nov. This is the only species of this genus, of which ten specimens are available.

# SUMMARY

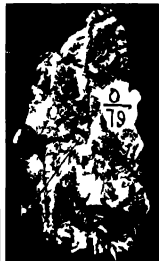
The forms described under the name *Ontheostrobus sessilis* come, as the name indicates, from *Onthea*. Most of the specimens show well preserved elongated receptacles bearing throughout their length numerous crowded

seeds. The seeds are sessile and placed in shallow cupules which in turn are seated on cushions the cushions being sessile on the receptacle. The receptacles from which the seeds have fallen off show the exposed surfaces of the cushions slightly hollowed out. A small pit in the centre of this hollow marks the position of the vascular supply of the seeds. The detached seeds usually found crowded together in their original groupings show an elliptical scar at their chalazal end this marks the area of attachment of the seeds with the receptacle. It is a noteworthy feature that not a single seed shows its micropylar end.

With the present data in hand it is not possible to express any definite views regarding the systematic position of this peculiar and interesting fructification. A distant comparison with the Bennettitales is however suggested.

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(ca  $3^1$ )



7 -)



8



9 (ca  $>2\frac{3}{4}$ )

1 to 6-9 *Ontheostrobis sessilis*

## EXPLANATION OF PLATES

All photographs are untouched They are of natural size except where the magnification is given The figured specimens are preserved in the Department of Botany and Geology University of Lucknow

## PLATE XIII

*Ontheostrobus sessilis* gen et sp nov (Photos 1-5)

- Photos 1-2 Groups of seeds exposed by their chalazal ends These seeds were attached all round the receptacle shown in Photo 3 0/77
- Photo 3 The receptacle showing a number of elliptical scars which were left by the seeds shown in Photos 1-2 0/77
- Photo 4 Portion of receptacle showing a number of elliptical scars Some of the scars show distinctly a small pit (marked *p*) in the centre probably marking the vascular supply of the seeds 0/79
- Photo 5 Counterpart of Photo 4 showing some of the seeds which were attached to the receptacle shown in Photo 4 One or two seeds show the presence of a distinct pit in the centre 0/79

## PLATE XIV

*Ontheostrobus sessilis* gen et sp nov (Photos 6-9)

- Photo 6 Portion of receptacle showing a number of seeds still attached to it It appears as if the seeds were enclosed in a shallow membranous cup like structure 0/81  $\times$  Ca,  $3\frac{1}{2}$
- Photo 7 A group of seeds exposed by their chalazal ends showing clearly the elliptical scar of attachment 0/78  $\times$  2
- Photo 8. Numerous seeds crowded together in their original grouping In the upper part of this photograph a small portion of the receptacle (*r*) is still left intact and shows a number of scars left by the seeds 0/78
- Photo 9 Portion of receptacle with the attached seeds 0/76  $\times$  Ca  $2\frac{1}{2}$

## SYMPOSIUM ON STATISTICAL METHODS IN PLANT AND ANIMAL BREEDING

A SYMPOSIUM on the application of statistical methods to plant and animal breeding was held on 26th and 27th December 1946 under the joint auspices of the Indian and National Academies of Sciences during their annual session at Allahabad. The proceedings of the Symposium were initiated by Sir C. V. Raman on the opening day of the session by introducing to the audience the speakers who had assembled for taking part in the discussion. Dr P. V. Sukhatme was in the chair. During the remaining sittings Mr K. Ramiah took the chair.

Dr V. G. Panse (Indore), opening the discussion, said that plant breeding formed one of the principal items of agricultural improvement everywhere. As an instance of the results achieved, he cited the sugarcane breeding at Coimbatore, pointing out that almost all sugarcane grown in India to day belonged to one or the other of the large number of Coimbatore varieties that had been produced. Improved varieties in certain other crops like rice, wheat and cotton had also spread over considerable areas. While ordinary crops were susceptible to damage from pests and diseases, improved varieties showing a high degree of resistance to diseases like wilt in cotton or rust in wheat had been bred and their cultivation saved millions of rupees worth of crop from loss. Again, as in cotton, improved varieties had better commercial quality than types previously grown. The important point about plant breeding was that the improvement in crop was brought about without any extra labour or expenditure on the part of the cultivator who had merely to grow the seed recommended to him in place of ordinary bazaar seed. At the present juncture when they were anxious to grow more food in the country, cultivation of improved varieties would go a long way in making good the deficiency.

Until not long ago, plant breeding was considered to be an art. It was not necessary now to go into the merits of this view because this view had gradually altered and plant breeding was to-day recognised as much a science as, for example, agronomy or engineering was. This transformation had been brought about largely as a result of the application of statistical technique in plant breeding. Text-books on plant breeding acknowledged this role of statistics by providing extensive appendices on statistical methods, but the speaker wished to emphasise that the part that statistics should play

in the day to day work of the breeder was more fundamental and more essential than was ordinarily imagined. The breeder should find use for statistics, not only after he had accomplished the main task of evolving an improved strain, as was often supposed but right through the whole process of breeding in order to direct his work along most effective lines. The speaker wished to present a brief review of the contribution that statistics would make to secure more rapid crop improvement.

The various problems in plant breeding could be divided into three groups. The first concerned the choice of material from which to breed. It was obvious that if improvement was to be effected in a crop there had to be scope for improvement. In statistical language improvement by breeding rested on the foundation of variability. Variability was of two kinds, genetic and environmental. In a field the individual plants differed in their performance with respect to any character in which the breeder might be interested partly on account of differences in their genetic constitution and partly due to differences in the conditions of their growth or environment. The variability observed in a crop whether in a commercial field or of hybrid origin, was thus a combination of both kinds. The environmental component of variability was, however, of no use to the breeder or rather hindered his work. If plants were selected for their superior performance, but if this superiority was mostly due to their better environment the performance of their progeny would not differ appreciably from that of the unselected plants. Only plants with superior genes in them gave progenies with high mean values. In terms of variability, it was the genetic fraction of observed variability that determined the capacity of a plant population to respond to selection. The breeder's choice of material for selection consequently depended on the amount of genetic variability it contained. It was however, not possible to measure genetic variability directly, because in any observational data, the genetic and environmental constituents were bound together indissolubly. Statistical methods had been developed for estimating the relative proportion of genetic variability in plant material (Panse, V. G., 1940, *Journal of Genetics* 40, 283). Taking as an example, the improvement of wheat in U.P. by selection, the breeder might collect bulk samples of seed from several distinct tracts in the province and grow these in a replicated varietal trial. Their comparison under a common environment would bring out samples with high mean values which would indicate their genetic superiority. Further, if the character under selection was measured in a large number of randomly selected plants in each sample in the trial, a measure of gross variability present in the character would be obtained. Out of the plants observed, progenies could

be grown from as large a number of randomly taken plants as could be managed, in a replicated progeny row test. Then the regression of the progeny means on the values of parent plants gave an estimate of the genetic fraction of variability observed in the parent population. Such a study thus provided two criteria which could guide the initial choice of material. These were mean values and genetic variability. Higher mean values assured a better mean performance in subsequent generations and greater genetic variability provided a larger scope for securing improvement over initial values. Where high mean value and large genetic variability went together the material was obviously the most suitable for selection. In samples where one of the criteria differed while the other remained substantially the same, that is, where variability differed but the means did not or *vice versa*, samples could be chosen on the basis of the variable criterion. But the problem presented a difficulty when both means and variability differed in opposite directions. A sample might have a low mean value but a high genetic variability or might have a high mean value coupled with low genetic variability. The question of preference between these two kinds of samples could not be decided on purely theoretical considerations. It was conceivable that even with a low mean value the variability might be so large that it could bring about by a suitable degree of selection improvement which would outstrip the improvement that could be attained by a similar degree of selection in a sample with a high mean value but low variability. The matter had to be examined experimentally. The speaker was studying at Indore the genetic variability in cotton samples collected from different parts of Central India and illustrated the point with results obtained in this study. Samples from 8 localities had been grown in a replicated varietal trial at Indore and about 150 randomly selected plants from all replications were examined for various characters, such as yield, earliness and quality in each variety. Replicated progenies from 30 random plants per variety were grown next season and the mean values of the progenies were correlated with parental values for estimating genetic variability. Results for fibre length which was the principal criterion for spinning quality in cotton, for three samples were given below.

Sample	Mean fibre length in mm in varietal trial	S.D. calculated from genetic component of variability
A	24.4	1.05
B	24.4	1.19
C	23.6	1.81

As far as samples A and B were concerned, sample A would be obviously the one to be preferred the means of the two samples were very much the same but there was greater genetic variability in sample A than in B. The comparison of the third sample with these two presented a difficulty, because sample C had a definitely low mean value but a much higher genetic variability. Starting with the means of the samples and assuming a normal distribution with the standard deviation given above it was easy to predict the improvement that would result from the selection of a certain proportion of the best plants, by calculating the average for the corresponding portion of the tail of the distribution. For one per cent selection, the calculated values were,

A	28.5
B	27.5
C	28.4

It was interesting to note that improvement in C in spite of its much higher variability, just reached the level of improvement brought about in A which had the advantage of a higher mean value. This led to the conclusion that between mean and variability, the mean was the more important criterion in the choice of material. He had obtained similar results in other cotton material consisting of a larger number of crosses in the *arborescens* species and it appeared from all these results that in material usually handled by the breeder, genetic variability would be rarely so high as to more than compensate for the initially low mean value and hence material with higher mean value was to be preferred when the choice had to be made both on mean value and variability. These views were however based on the speaker's experience with cotton and a different situation might exist in some other crops. It was of utmost importance that breeders and statisticians should co-operate in examining other plant material in a similar manner in order to build up a body of information which would provide guiding principles in the choice of suitable breeding material.

If the conclusion that the choice should primarily rest on mean value was general in its scope, it led to some important and interesting consequences in relation to the stage at which selection should be commenced in hybrid material. Breeders started selection in such material only in the segregating generations such as  $F_2$  or  $F_3$  or frequently even later. If selection were to be based both on variability and mean it would be necessary to grow the  $F_2$ s of the crosses which were to be compared but if mean values alone were to be taken into account the comparison could be made at the  $F_1$  stage and only such of the crosses which had the highest mean values could be retained for further selection with them. Breeders would

easily appreciate what immense saving of time, labour and expense this would mean, and these could be diverted to a more intensive examination in the subsequent generations of the few crosses chosen in the  $F_1$ . Immer had made a study of certain barley crosses through four generations and had reported that  $F_1$  comparisons remained substantially unaltered in the subsequent generations (Immer, F. R., 1941, *Jour. Amer. Soc. Agro.*, 33, 200). A question might be asked whether it was not possible to go a stage further back and compare the parent strains themselves for making crosses in which selection was to be made. The performance of  $F_1$  could not be predicted accurately from the values of the parents concerned, because it could be shown theoretically that even parents with individually low values could produce hybrids exceeding those from parents with superior values. In actual hybrid material extending over some 60 crosses in cotton (*arborescens* species), the speaker had found a correlation of 0.86 between  $F_1$  and  $F_2$  means but the correlation between  $F_2$  mean and the average of the two parents was 0.75. Comparison of crosses by means of the  $F_1$  therefore appeared to be a sounder procedure. As far as mean values were concerned selection in  $F_1$  was as good as in  $F_2$  on account of the strong correlation between the two.

The second group of the plant breeders' problems concerned the actual process of selection. In the early days breeders depended on what was termed 'mass selection'. The method was to collect the produce of suitable plants in the field, bulk the produce, raise the next year's crop from this produce, reselect suitable plants in this crop and grow them in bulk again. The process was repeated until a bulk with better performance than ordinary seed was obtained. The method of mass selection was replaced later by 'progeny-row-breeding' which was found superior because mean values of progenies grown separately from the seed of single plants gave a more dependable criterion of their genetic potentiality than the observed values of individual plants. This could be demonstrated by a statistical reasoning.

Supposing that there was a group of plants belonging to  $n$  progenies each consisting of  $k$  plants, there would be a total of  $nk$  plants. The genetic component of variability in this group of plants might be designated as  $g$  and the environmental component by  $e$ . If the  $n$  progenies were grown separately and assuming no genetic variability within a progeny the analysis of variance of the results could be set down as

Due to	Degrees of freedom	Variance
Progenies ..	$n-1$	$kg + e$
Plants within progenies ..	$n(k-1)$	$e$

The variance between progeny means would be  $\sigma^2/k$  while that between individual plant values within progenies would be  $\sigma^2$ . If the material were grown as a bulk of  $nk$  plants, not keeping the progenies distinct, the total variance would be  $\frac{k(n-1)}{nk} \sigma^2 + \sigma^2/k$ . The coefficient of  $\sigma^2$  was a fraction less than 1, except that this coefficient would be equal to 1 when  $k$  was equal to 1. Comparing the variances for progeny means and for the bulk it was clear that in progeny row breeding the genetic component formed the dominant fraction of variability whereas in mass selection the environmental component would be the larger fraction, except where the bulk was constituted by taking a single plant per progeny in which case the two fractions would be equal. In either case mass selection would be less effective than progeny row breeding being subject to a greater influence of environment.

It should be noted however, that even progeny means were affected by a fraction of environmental variability,  $\sigma^2/k$  in the above analysis and numerical differences between progeny means could not be used as a sufficiently reliable guide to genetic differences. A critical decision on this point could be made only by growing progenies in replication and using the standard error for comparison of observed differences in progeny means. It was in the matter of making replicated tests of progenies that considerable improvement was needed in the present-day methods. Various experimental designs particularly adapted to plant breeding material had been developed for this purpose and it was encouraging to find a growing realization of the value and utility of these designs on the part of plant breeders. It was not necessary to enter into details concerning these designs here.

Though selection was now primarily based on progeny means, the desirable progenies had to be propagated from selected plants. This was the weakest point in the selection procedure because single plant values which were susceptible to a large degree of environmental variation had to be employed to represent the genetic constitution of superior progenies. With progenies grown in replication, single plants could be selected with greater confidence if the selection was based not on the observed value of a plant but on the deviation of this value from the mean of the plot. This would avoid undue weightage being given in selecting plants to those replicates which had given a higher performance owing to their more favourable location. Another approach to this problem was made possible by the discriminant function technique. In selecting plants for yield, for instance, the usual method was to select those plants that gave a higher yield. A second alternative was, to consider the components of yield and select with the help of a weighted linear function of these components the weights depending on

the relative susceptibility of the individual components to environmental fluctuation and their mutual correlation. The yield of a wheat plant, for example, was the product of the number of ears, number of grains per ear and weight per grain. The yield of a cotton plant was similarly made up of the number of bolls per plant, number of seeds per boll and the weight of seed cotton per seed. The environmental and genetic variabilities of these components could be estimated by growing a number of varieties or lines in a replicated experiment and measuring the component characters in each plot. With these data the discriminant function technique enabled the evaluation of a formula in terms of the observed values of the component characters appropriately weighted and giving a numerical score most highly correlated with the genetic capacity of a variety or line. It was difficult to state in the absence of more experimental studies how much practical use the method had in helping single plant selection. The speaker's own conclusion from investigation on cotton and wheat at Indore was that when only components of the characters to be selected for were taken into account, the discriminant function did not appear much superior to straightforward selection on the character itself, but the discriminant function had the advantage in permitting selection to be based on characteristics which, though not components of the character to be selected, might be highly correlated with the latter. An example was provided by tillering in wheat which though not a direct component of yield was highly correlated with it and could be included in the discriminant formula thereby making selection for yield more efficient.

The third group of problems that the breeder had to tackle related to the maintenance of superior strains once these had been evolved. It was a common experience that such strains tended to deteriorate or run out in the course of time. Various causes were responsible for deterioration. One of them was mechanical admixture with inferior seed. In cotton, the seed was obtained from ginneries which were the principal source of mixture between varieties. Mixture might take place in the field also. Then there was cross-pollination with other varieties. A third factor responsible for deterioration was the residual genetic variability left in the strain. Although genetic variability was rapidly lost with the progress of selection a residue might persist when the strain was given out for commercial cultivation and this might lead to segregation of inferior genotypes and their spread in the strain. The breeder was aware of the necessity of maintaining the strictest possible purity in the nucleus seed, but he generally concentrated on morphological purity and in fact did not employ any means for testing the degree of purity in characters such as yield or quality for which the strain

was bred. The usual method of collecting selfed seed from typical looking plants for further propagation did not permit such a test being made. The proper method for this purpose was to maintain the strain by growing a replicated progeny row trial each season. In such a trial the possible beginning of deterioration could be detected at an early stage and suspected progenies could be discarded before bulking the rest for providing the nucleus seed. Elimination of inferior progenies would be made easier by making the test of significance less rigorous than the usual 5 per cent probability level and it was to meet this need that tables of 'z' and the variance ratio for 10 per cent level had been prepared (Panse, V. G., and Ayachit, G. R., 1944, *Ind Jour Agric Sci*, 14, 244).

The speaker concluded his remarks with a reference to the need for a study of quantitative genetics. The earlier expectation that genetics would revolutionize plant breeding had not been borne out mainly because genetical investigation had been restricted to the formal mendelian characters while the study of quantitative characters which were the ones with which the breeder was really concerned had lagged far behind. This study could be pursued with the help of statistical methods coupled with adequate experimental material and provided a very rich field for co-operative work by Statisticians, Geneticists and breeders. The contribution that genetics had to offer to plant breeding depended on the advance in our knowledge of the behaviour of quantitative characters.

Dr P. V. Sukhatme (Delhi), who led the discussion on the animal breeding side, said that a clear exposition of the rôle of statistical methods in plant breeding had been given by Dr Panse. As the subject of symposium also included the rôle of statistics in animal breeding, he proposed to confine his remarks to the latter.

At the outset Dr Sukhatme said that although an enormous change had been brought about in the plant-breeding methods as a result of the work of Dr Panse and his colleagues at Indore, in animal breeding, they were a long way off from that ideal. The methods of animal breeding when contrasted with those of plant breeding were remarkably speculative and continued to be so in spite of the great progress in statistical science. This Dr Sukhatme attributed to several factors. He said that whereas plants could be reproduced and multiplied many-fold every year, it took two to three years and even more to reproduce another generation of animals and five to six years for a group of animals to double their number. Then again whereas in agricultural crops the improvement could be effected by a few specialists from whom improved seeds could be bought and multiplied, the

improvement in animals rested with a large number of animal owners. Success in plant breeding had been attained by substituting the system of selection in which the value of an individual plant was judged by the average of its progeny for a system in which it was judged according to its individual performance. This, however, was not easy to accomplish in animal breeding. Then again an animal was relatively far more costly to experiment with than a single plant. For these reasons, he said the methods in animal breeding stood relatively still in contrast to those adopted in plant breeding.

Despite these inherent limitations, Dr Sukhatme said that statistical methods could play a valuable rôle in helping animal breeders to attain their aims. He said that he would illustrate this by describing a breeding project on goats financed by the Imperial Council of Agricultural Research.

The scheme was started with a foundation stock of 47 does and 4 bucks with the object of improving the average milk yield per day of lactation and of the kidding interval. It had been in progress for 10 years, when, he said, he was called upon to assess the progress made. He presented Table I.

TABLE I  
*Comparison between the performance of the foundation stock and their first progeny*

		Foundation stock				First progeny			
		No of goats	No of lactations	Total milk yield (oz.)	Length of lactation days	Average milk yield per day of lactation (oz.)	No of goats	No of lactations	Length of kidding interval days
Foundation stock	Average Standard Error	19	68	4761.8 198.5	176.7 8.4	26.9 1.8	16	48	312.5 16.9
First progeny	Average Standard Error	34	130	6812.5 210.3	176.1 5.2	33.1 1.4	90	110	291.4 9.5
t				3.31	0.1	2.9			1.1
									3.51

showing the comparison between the performance of the foundation stock and that of its progeny and said that the progeny had given a significantly higher milk yield during per day of lactation and of kidding interval. This might not, however, be due wholly to the breeding policy followed on the farm. It might have resulted, for instance, from the better feeding and management right from the birth of the first progeny in contrast with the environment in which the dams were reared. Hybrid vigour might also partly offer an explanation. There might also be seasonal effect arising from the different climatic and disease conditions in the different years in which the dams and their daughters had their respective lactations.

He then presented a second table showing sire-wise comparison of the first progeny and their mothers and showed that the performance of the first progeny over their dams was significant in the case of sire No 48 M alone and not in others. But even in the case of sire 48 M, he said, he could adduce evidence to show that the better performance of the first progeny was, to a very considerable extent, due to better feeding and management on the farm, to differences in the years in which the majority of the lactations of the foundation stock and the first progeny were completed and to the preponderance of winter kiddings in the first progeny. It was, however, difficult, to make any exact allowance for these factors. The best he could do was to compare the relative worths as transmitters of milk yield of bucks 48 M and 49 M as this comparison was free from consideration of the order of lactation or seasonal effects. The values of  $t$  of this comparison given in the last row of Table II indicated that while there was

TABLE II  
*Progeny tests for bucks 48 M, 49 M and 50 M*

		No. of goats	No. of lactation	Total milk yield (oz.)	Length of lactation days	Average milk yield per day of lactation (oz.)	No. of goats	No. of lactations	Length of kidding interval day	Average milk yield per day of kidding interval (oz.)
		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
48M Foundation stock	Average	11	38	4498.1	173.6	25.9	9	21	299.5	17.5
	Standard error			302.3	12.5	2.3			20.8	1.7
First progeny	Average	14	55	6199.5	178.2	34.8	12	15	246.9	23.2
	Standard error			483.8	8.1	4.4			5.2	0.8
$t$				3.4†	0.3	2.8†			0.5	3.2†
49M Foundation stock	Average	0	25	4392.7	161.4	27.2	5	18	293.4	15.4
	Standard error			260.1	10.3	3.5			20.6	2.4
First progeny	Average	8	35	4690.7	157.0	31.3	7	31	268.6	18.2
	Standard error			340.8	7.6	3.0			15.2	2.0
$t$				1.1	0.3	0.0			1.0	1.0
50M Foundation stock	Average	1	3	5702.0	197.3	28.9	1	2	302.5	22.6
First progeny	Average	1	5	5891.2	197.4	35.2	1	4	337.5	17.0
$t$				Values of $t$ for the comparison of the bucks 48M and 49M						0.8
				1.8						
				0.7						

136 *Symposium on Statistical Methods in Plant & Animal Breeding*

a suggestion that buck No. 48 M was probably superior to 49 M, there was no adequate evidence to establish his superior worth over the other.

He next presented the results of the comparison of the second progeny with their mothers in the first progeny and those of the third progeny with their mothers in the second progeny and said that, far from showing any improvement, the results indicated a steady deterioration in the performance

TABLE III

*Comparison between the performance of the second progeny and their dams*

		No. of goats	Total milk yield (oz.)	No. of days in milk	Average milk yield per day (oz.)	No. of goats	Kidding interval	Average milk yield per day of kidding interval (oz.)
First lactation	1st progeny Average	18	5396.3	167.8	32.2	18	264.3	20.5
	Standard error		423.6	11.9	1.1		23.1	1.2
	2nd progeny Average	27	4864.1	170.9	27.6	25	313.7	14.8
	Standard error		349.7	11.0	0.9		23.7	1.2
<i>t</i>			0.9	1.7	3.2†		1.4	3.6†
Second lactation	1st progeny Average	15	5558.4	162.6	36.4	11	237.1	23.6
	Standard error		131.4	8.8	1.4		22.8	1.3
	2nd progeny Average	15	5216.0	192.0	27.2	12	0.0	15.7
	Standard error		102.5	9.8	1.4		3328.7	1.3
<i>t</i>			0.6	3.0†	4.8†		1.9	3.8†
Third lactation	1st progeny Average	7	7006.3	206.0	38.4	Sufficient data not available		
	Standard error		1418.6	28.2	2.1			
	2nd progeny Average	7	5617.4	215.7	26.0			
	Standard error		931.3	29.6	2.2			
<i>t</i>			1.4	0.2	4.1†			

TABLE IV

*Comparison between the performance of the third progeny and their dams*

		No. of goats	Total milk yield (oz.)	No. of days in milk	Average milk yield per day (oz.)	No. of goats	Kidding interval	Average milk yield per day of kidding interval (oz.)
2nd progeny Average	..	4	4703.5	148.3	31.7	4	256.5	18.4
	Standard error		950.3	13.8	2.7		39.3	3.4
3rd Progeny Average	..	8	5280.9	227.3	23.2	7	392.3	13.4
	Standard error		658.7	34.0	1.1		64.3	1.3
<i>t</i>	..		0.4	2.0	3.0†		1.4	1.5

TABLE V  
Effect of selection in the foundation stock

Character compared		Foundation stock dams				First progeny daughters			
		No. of goats	No. of lactations	Value (oz)	Standard error (oz)	No. of goats	No. of lactations	Value (oz)	Standard error (oz)
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Total milk yield oz.	First Rest	9 10	35 33	5385.6 3867.6	216.3 265.1	15 19	68 62	5683.7 6995.7	286.5 310.4
<i>t</i>	**			5.1				0.7	
Average milk yield per day of lactation oz.	First Rest	9 10	38 30	30.3 22.8	2.3 2.0	10 18	63 67	32.8 33.6	1.9 2.1
<i>t</i>	**			2.6*				0.2	
Average milk yield per day of kidding interval oz.	First Rest	8 8	25 23	22.1 12.2	0.7 0.9	14 16	56 54	21.5 20.4	1.3 1.0
<i>t</i>				7.9†				0.7	

of both the second and the third progenies. He also presented the results of progeny tests for individual bucks giving similar conclusions. It was clear, he said, that while the first progeny showed better performance, there was a steady deterioration in the performance thereafter.

Dr. Sukhatme next considered the question whether selection from amongst the dams would have led to improvement in the successive progenies. An examination of this question was made possible because no culling of the female progeny was practised as a part of the policy followed on the farm. The whole of progeny was kept and bred up. For this purpose, he said, the mothers of the foundation stock were ranked in order of their performance and the results of the performance of the progeny of the first half were compared with those of the rest as in Table V. The results showed that while the performance of the second half of the foundation stock was significantly different from that of the first half, their progenies did not show a significant difference in the performance. He had similarly compared the results of the first and the second progeny and had reached identical conclusions, *vide* Table VI. It was clear, he said, that selective breeding on the side of the dams was without effect thus suggesting that there was no correlation between the performance of the dams and their daughters,

TABLE VI  
Effect of selection in the first progeny

	Character compared		1st progeny dams		Standard error (oz)	2nd progeny daughters		Standard error (oz)
			No of goats	Value		No of goats	Value	
First lactation	Total Milk yield (oz)	First Rest	3	161.6	3.9	17	4827.0	414.0
			6	171.1	7.0	10	4927.2	136.8
	<i>t</i>			1.41			0.1	
	Average milk yield per day of lactation (oz)	First Rest	9	7.7	1	1	27.3	1
			1	28.5	1	14		1.0
	<i>t</i>			1.8			0.2	
Second lactation	Average milk yield per day of kidding interval (oz)	First Rest	3	24.6	1	1	14.7	2.0
			3	16.1	0	13	12.0	1.3
	<i>t</i>			5.4			1.1	
	Total milk yield (oz)	First Rest	7	6881.4	213	8	6929.7	111.1
			9	4498.8	180.1	8	3226.1	14.1
	<i>t</i>			4.8			1.1	
	Average milk yield per day of lactation (oz)	First Rest	7	40.7	1.7	7	24.2	1.6
			8	32.7	0.1	5	9.8	1.6
	<i>t</i>			1.91			2.5	
	Average milk yield per day of kidding interval (oz)	First Rest	5	16	1	6	16	2.4
			1	20.5	1	6	17.1	1.0
	<i>t</i>			1.2			0.4	

TABLE VII  
Coefficients of correlation between the performances of dams and daughters

	Total milk yield (oz) (1)	Average milk yield per day of lactation (oz) (2)	Average milk yield per day of kidding interval (oz) (3)
Foundation stock and first progeny	0.170	-0.270	-0.012
First progeny and second progeny first lactation	0.171	-0.005	0.064
First progeny and second progeny second lactation	-0.168	-0.397	-0.266

This, he said, was actually the case, as would be seen from the values of the coefficient of the correlation in the accompanying Table VII. These results

indicated that the foundation stock had a low genetic variability. He was rather puzzled to find that certain coefficients were negative but, he said, they were not significant. It was possible, he added, that some of the assumptions underlying the estimation of genetic variability from the regression of the progeny means on the parental values were not realized in practice.

He concluded by saying that had the progeny tests been carried out in time the results would have revealed a prepotency of the different sires and suggested suitable changes in regard to their use. As it was, he observed, four to five valuable years were wasted in trying to locate without success superior bucks by basing judgment on conformation instead of on the results of the progeny tests. Similarly a study of the genetic variability in the successive generations would have revealed the potentiality for improvement of the material and suggested suitable breeding plans. Yet another factor contributing to the lack of progress was the use of too few bucks to start with. He was glad that now, at any rate, the animal breeders had realized the potentiality of statistical tools as an help in attaining their objectives and were freely seeking the help of the Statistical Section of the Imperial Council of Agricultural Research in their work.

Mr K. Kishen (Lucknow) said that it was the function of the plant breeder to evolve out crop varieties giving high yield, of superior quality, resistant to disease, to lodging. For that purpose, the breeder had to experiment with a large number of plants or strains which he obtained by various methods of selections. Initially, the number of plants he had to select from was large. On account of the inadequacy of the seed material it was not possible to lay out randomised replicated trials at this stage. In making selection for the most promising varieties and the most promising progenies of a variety, the breeder had to take into consideration a number of quantitative characters for each plant such as yield of grain per plant, ear number per plant, average number of grains per ear, and to select plants from the point of view of the requisite character or quality of the crop, i.e., yielding capacity. Till the advent of statistical methods in plant breeding, this selection was essentially subjective and was attended with the risk that some promising strains or progenies might be rejected and undesirable plants or strains might be selected by this process. This selection was now best done by applying the method of the discriminant function.

Mr Kishen then gave a brief outline of the method of the discriminant function and pointed out that selection by this method maximised the expectation of the genetic advance.

Further he observed that after the preliminary selection by the discriminant function the breeder was still left with a considerable number of varieties and progenies of those varieties. For the selection of the best varieties and progenies of those varieties, it was necessary to conduct replicated progeny row trials and lay out compact family-block designs. In those designs with a large number of varieties the size of block would become too large and require reduction in order that the elimination of fertility differences might be done efficiently. As a result of recent researches in the design of agricultural experiments, a large number of designs were available which brought about the desired reduction in the size of the block in such trials. Those were known as incomplete block designs, because, unlike the ordinary randomised block designs, the number of plots per block was less than the number of varieties, in these designs.

Let  $b$  be the number of blocks,  $k$  the number of plots per block,  $v$  the number of varieties,  $r$  the number of times a variety was replicated within a block and  $\lambda_{ij}$  the number of blocks in which  $i$ th and  $j$ th varieties occurred together. Such a general design was known to be analysable if it was a connected design.

He then briefly explained the concept of connectedness introduced by R. C. Bose, and cited the following two-dimensional square lattice in two equal groups of sets for 9 varieties as an example of an incomplete block design which was connected.

Block I	1	2	3	1	4	7	Block IV
Block II	4	5	6	2	5	8	Block V
Block III	7	8	9	3	6	9	Block VI

He explained how in this case one could pass from any one variety to any other, say 1 to 9, by a chain of varieties and blocks like 1, I, 3, VI, 9 so that the design was connected. He said that it was interesting to note that a two-dimensional square lattice in one group of sets did not give a connected design.

If  $V_1, V_2, \dots, V_v$  denoted the true varietal effects, then  $\sum_{i=1}^{v-1} l_i$ , where  $\sum_{i=1}^{v-1} l_i = 0$ , was termed a varietal contrast. It was known from the theory of linear estimation that in a connected design every varietal contrast was estimable. Thus a connected design was analysable. He mentioned several examples of connected designs and added that the statistical theory of design of experiments had now advanced to such an extent that it was possible to provide the plant-breeder with an appropriate design which he

may require under any circumstances for laying out a suitable compact family block trial and the parallel bulk trial

He further, observed that the number of varieties and the progenies selected by the above procedure would be small. It would then be necessary to conduct simple randomized block experiments with those varieties at a number of stations for about three consecutive seasons before the varieties which were most suitable for distribution among the cultivators were finally decided upon.

Referring to the statistical methods in animal breeding he said that the technique of animal breeding was in its initial stages not essentially different from that of plant breeding. Selection by the method of the discriminant function was advantageous in animal breeding also. As an example he referred to a recent paper entitled "An application of the discriminant function for selection in poultry" by Dr V. G. Panse (*Journal of Genetics*, 1946, 47, 242) and pointed out that as a result of the use of the discriminant function in this case, the percentage of genetic advance over straight selection varied from 10 to 30 per cent.

He remarked that animal nutrition experiments also formed a part of the science of animal breeding. In those experiments the statistical principles of replication and randomization were extensively employed. In some cases, litters of animals, such as rats, had to be dealt with. In such cases, litters corresponded to blocks and animals within a litter to plots and the appropriate connected design, which in the simplest case might be a randomized block design, could be laid out for testing the efficacy of nutritional treatments.

He concluded by saying that it was sometimes necessary to test the efficacy on animals of several interacting groups of factors e.g. dietary treatments. In such cases it was appropriate to lay out a factorial design. But usually the main difficulty in laying out a factorial arrangement in those cases was the inadequacy of the number of animals available for experimentation. That difficulty had been largely overcome by the device of fractional replication of a factorial arrangement, introduced by D. J. Finney, and extended since by the speaker himself. He illustrated the theory of fractional replication by considering the  $2^4$  factorial design, where there were 16 treatment combinations, but where replication of half, i.e. eight, of the combinations could yield valuable information regarding main effects and the more important interactions. Thus, factorial designs with fractional replication, in that they enabled factorial experiments to be conducted with one-half, one-third, etc., of the total number of treatment

combinations were likely to be very useful for exploratory experiments in animal nutrition

Mr V D Thawani (Delhi) said that he would deal with the discriminant function once more although Dr Panse and Mr Kishen had referred to it earlier, since it was a very useful instrument of plant selection. He pointed out that the equations from which Mr Kishen had started were  $X = \sum b_i x_i$  and  $\phi = \sum a_i \eta_i$ , where  $x_i$  and  $\eta_i$  were the phenotypic and genotypic values, and the  $a_i$ 's were given and  $b_i$ 's were chosen in such a way that correlation coefficient  $\rho$ , between  $X$  and  $\phi$  was the greatest. This could be looked upon from a different angle as well. The idea of plant selection was that the mean genetic value of the selected part should be as much as possible in excess of the mean of the unselected population. This excess might be called the genetic advance. If they had a large number of progenies and they had to select one  $q$ th part of them for further propagation, they had to maximise  $E(\phi) - \bar{\phi}$  where  $E(\phi)$  was the expected value of the selected part and  $\bar{\phi}$  was the mean value of the whole unselected population. It could be shown that that was equal to  $\frac{z}{q} \sqrt{\text{Var } \phi} \rho$ , where  $z$  was the ordinate marking off the one- $q$ th part of the area of the standardised normal curve. Thus maximisation of  $\rho$  was the same thing as maximisation of  $E(\phi) - \bar{\phi}$ . Thus selection was based on the idea of the greatest genetic advance. It was understood that the one- $q$ th part of the progenies which were selected for further propagation corresponded to the highest phenotypic values  $x_i$ .

His second point was that  $b$ 's were expressed in terms of  $a$ 's and certain variances and covariances whose estimates were obtained from the analyses of variance and covariance. In that connection he asked them to consider in particular the equation  $x_i = \eta_i + \xi_i$ , where  $x_i$ , the phenotypic value, was made up of two parts  $\eta_i$  the genotypic value and  $\xi_i$ , the component due to environment. There it was assumed that the two parts were additive, which gave

$$\text{Var}(X_i) = \text{Var}(\eta_i) + \text{Var}(\xi_i)$$

$$\sigma^2 = \sigma_{\eta}^2 + \sigma_{\xi}^2 + f, \text{ (say)}$$

if  $\text{Cov}(\eta_i, \xi_i) = 0$  i.e., the two parts  $\eta_i$  and  $\xi_i$  were assumed to be independent

Then the analysis of variance gave the result

Source of variation	Mean square
Between progenies	A
Within progenies	B

Obviously, B was an estimate of  $f$  and A of  $d$ , and therefore A-B was an estimate of  $e_{ii}$  since  $d_{ii} = e_{ii} + f_{ii}$ . If A-B happened to be negative in practice, they took  $e_{ii} = 0$ . That led to a difficulty and it appeared advisable to look into the assumptions of additiveness and independence of genotypic and environmental components, on which the derivation of estimates of genotypic and phenotypic variances was based. He expressed doubt about the correctness of those assumptions and suggested that the operation law might be analogous to the vector law in place of the simple additive law.

Coming to the next point he asked them to consider the equation

$$X = \sum b_i x_i$$

If one of the characters, say  $x_1$ , was unimportant and was dropped,  $a$  might be put equal to zero. In that case the ratios of  $a_i$ ,  $a_j$  changed thus changing the values of  $b$ 's altogether. The recalculation of  $b$ 's involved heavy computation and it might be possible to define functions of  $b$ 's (say linear) which did not change even if any one of the characters was finally dropped. This point needed to be looked into mathematically.

Then he came to his last point and said that the discriminant function X was taken as a linear function of  $x_i$ 's, viz  $X = \sum b_i x_i$ . He asked if it was possible that a non-linear function,

$$X = \sum b_i x_i + \sum b_{ij} x_i x_j + b_{ijk} x_i x_j x_k + \dots$$

might give a better result than a linear one, and if in such a case it was possible to define  $b$ 's in such a way that they remained unaltered even when only the linear terms were taken.

Mr S. D. Bokil (Indore) considered theoretically the relationship between hybrid vigour and  $F_1$  and  $F_2$  variances in the light of a simple Mendelian scheme of factors. He said that the following assumptions were made regarding the inheritance of quantitative characters, such as staple length in cotton.

- (i) Such characters were affected by a large number of independent factors each having individually small effect.
- (ii) The factors had no epistatic relationships.
- (iii) They had equal effect on the particular character.

The justification for these assumptions was, firstly, that they were simplifying, and secondly that the assumption (i) was necessary to explain continuous variation of such characters while assumptions (ii) and (iii) gave results approximating to those obtained without them (Fisher, R. A., 1918, *Trans. Roy. Soc. Edinb.*, 52, 399; Panse, V. G., 1940, *Journal of Genetics*,

40, 283) Under these circumstances the means and variances obeyed the additive law, i.e., they were the algebraic sums of contributions derived from individual factors

He then proceeded to consider the crossing of two pure varieties, i.e., varieties homozygous for all factors concerned. Let one variety contribute  $p$  factors not contributed by the other favouring big size and other variety  $q$  similar factors. Only such factors needed to be considered since factors common to both the parents could not have any effect on hybrid vigour or variances of subsequent generations. It was obvious that the  $F_1$  generation would be heterozygous for  $p + q = n$ , say, factors

The three phases of any factor could be denoted by  $d$ ,  $h$  and  $d$ . If there was no dominance  $h = 0$  and the three phases would be denoted by  $d$ ,  $0$  and  $d$ . The expectation of the mean of the  $F_1$  generation would be  $0$  in that case. On the other hand, if all factors were fully dominant for big size the sum  $\sum h = nd$  or  $n$  if measurements be made in units of  $d$ , would represent  $F_1$  value. If 3rd of the factors were fully dominant for big size and 4rd for small size the sum  $\sum h$  and hence the mean of  $F_1$ , would be  $0.33n$

The  $F_2$  variance in the corresponding cases could be calculated by calculating the contribution of a single factor to  $F_2$  variance. For each factor,  $\frac{1}{4}$ th of the progenies were homozygous for each of the two allelic phases and  $\frac{1}{2}$  heterozygous, the respective phenotypic values being  $d$ ,  $d$  and  $h$ . The mean would be  $\frac{h}{2}$ , hence variance due to single factor would be

$$\frac{1}{4} \left( d - \frac{h}{2} \right)^2 + \frac{1}{2} \left( h - \frac{h}{2} \right)^2 + \frac{1}{4} \left( d + \frac{h}{2} \right)^2 \\ = \frac{1}{4} (2d^2 + h^2)$$

as had been shown by Fisher, Immer and Tedin (Fisher, R. A., Immer, F. R., and Tedin, Olof 1932, *Genetics*, 17, 107). Hence the  $F_2$  variance due to  $n$  factors would be  $\sum \frac{1}{4} (2d^2 + h^2) = \frac{1}{4} nd^2 = 0.25n$ ,

for no dominance, i.e.,  $h = 0$ . Similarly for complete dominance variance in  $F_2$   $\sum \frac{1}{4} (2d^2 + h^2) = \frac{3}{4} nd^2 = 0.75n$

for both the subsequent cases where dominance is either in one direction only or in both directions since  $h^2 = d^2$  in both cases. He considered some

other cases and by similar methods calculated the mean variance within and between  $F_2$  progenies also and presented the following table—

type of dominance	$h_1$ (Hybrid vigour)	$V_{F_2}$	$V_1$ (between progenies)	Max. Genotype
No dominance	0	.50 $n$	.50 $n$	$n$
Full Dominance—				
(i) All factors for big size	$n$	.75 $n$	.75 $n$	$n$
(ii) $\frac{1}{2}$ for big, and $\frac{1}{2}$ for small size	.33 $n$	.75 $n$	.60 $n$	$n$
Partial Dominance—				
(i) $h = .5$ for all factors	.5 $n$	.60 $n$	.52 $n$	$n$
(ii) $h = .8$ do	.8 $n$	.68 $n$	.54 $n$	$n$

The mean variance within  $F_2$  progenies was exactly half the variance in  $F_2$  and could be calculated from the above table. All cases had the same maximum genotype indicating the same degree of maximum improvement attainable by selection. It was apparent from the table that with a given number of genes and with the same type of dominance, the variance in  $F_2$  was higher for a larger value in  $F_1$  and consequently even if variances as well as mean values were to be taken into account in selection, selection in  $F_1$  would be reliable, as  $F_1$ 's with higher values could also be expected to give larger variances in subsequent generations. If in actual experimental material this association between  $F_1$  values and  $F_2$  variances was not realized, a possible explanation was provided by the case for balanced dominance shown in the table. There the  $F_1$  with a value lower than all other cases of dominance gave an  $F_2$  with the largest  $F_2$  variance. It had to be noted, however, that even here the  $F_2$  variance was not larger than that obtained with full dominance for all factors which also had the highest  $F_1$  value.

The large genetic component of  $F_2$  variance between progenies as well as its approximate constancy in the different cases suggested that selection might be profitably exercised in  $F_2$  generation, but, the expense of growing a large number of  $F_2$  progenies would be considerable. The relative efficiency of selection in different generations was required to be studied carefully by weighting the considerations of expense and the improvement to be expected appropriately.

Mr. R. S. Koshal (Poona) said that there were three well-known methods of plant improvement, namely, importation and subsequent acclimatisation of foreign seed, mass or single line selection, and hybridization. He proceeded to describe the third method and to show how selection from suitable crosses could be made in  $F_1$  generation without going further to the  $F_2$  stage, thus saving considerable time and labour. Here the statistical methods had helped the plant-breeders to evolve a suitable technique

and the essential requirement of that technique is that the parents and the  $F_1$ 's should all be grown the same year in a randomized replicated trial. Material for such a study was available from an experiment which had been carried out at the Institute of Plant Industry, Indore, and he illustrated the method with reference to three varieties and their  $F_1$  crosses, which had been grown in the randomized blocks.

The varieties could be denoted by AA, BB and CC and their  $F_1$ 's by AB, AC and BC where A, B and C represented the groups of genes in the parents responsible for fineness. There were 5 degrees of freedom for comparison among the parental varieties and their  $F_1$ 's. These could be split up into 2 for the genetic part, 1 for heterosis and 2 for epistacy or interactions between genes. The genetic part gave comparisons between the three expressions  $2AA + AB + AC$ ,  $2BB + BC + AB$  and  $2CC + CA + BC$ , and these comparisons showed which variety was better for crossing. The comparison between the mean of the parents and their  $F_1$ 's provided evidence for heterosis or hybrid vigour. The comparison was of the type

$$\begin{aligned} &AA + BB - 2AB, \\ &AA + CC - 2AC, \\ &BB + CC - 2BC \end{aligned}$$

The sum of these comparisons for the three crosses contributed a single degree of freedom for general heterosis. The remaining 2 degrees corresponding to epistacy consisted of comparisons between the quantities,  $AA + 2BC$ ,  $BB + 2AC$ , and  $CC + 2AB$ . In the material under consideration it was found on the above analysis that the variety B which was Bani (*G. arborum forma Indica*) was superior to the other two varieties, Malvi and Cwn 520, for crossing. This illustrated how in some cases it was possible to eliminate undesirable material at  $F_1$  stage if a suitable technique was employed.

He further stressed that as already pointed out by Dr Panse, plant variability can be traced to two causes: (a) Environmental, (b) genetic, and it is the latter which plays an important rôle in plant selection work. For this purpose it is essential that plant-breeder should grow his material in replicated blocks and make selection on the basis of higher mean value and higher regression of progeny means on parental plants.

Mr V. B. Sahasrabudhe (Indore) after referring to the importance of replicated progeny row trials emphasised by earlier speakers, said that standard layouts adopted for such trials consisted of randomised blocks with plots consisting of 5 to 10 plants replicated 5 to 10 times depending

upon the quantity of seed available. The method of growing progenies in strips with controls after 10 to 15 progeny rows was, however, adopted by some breeders. For a large number of progenies the various incomplete block designs would appear to provide suitable layouts.

He then discussed the results for two plot sizes  $6' \times 2'$  and  $12' \times 2'$  from a uniformity trial which was laid at the Institute of Plant Industry, Indore, with an Institute bred cotton strain, Dhar 43. The spacing between rows was two feet and between plants one foot, this being the standard spacing adopted for plant breeding trials at Indore. The results consisted of the following comparisons.

Comparison of the relative efficiency of simple randomised blocks with (1) strips with controls at regular intervals, (2) compact blocks with controls at regular intervals, (3) various incomplete block designs was shown in the following table:—

*Relative efficiency of simple randomised blocks with strips and compact blocks with controls*

Plot size	No. of progenies	Relative efficiency (Efficiency of randomised blocks = 100)	
		strips	Compact block with controls
$6' \times 2'$	30	73.2	83.2
	40	72.8	83.8
	50	75.2	83.2
$12' \times 2'$	30	82.4	83.0
	40	77.7	7.5
	50	83.1	83.0

Both the designs were less efficient than simple randomised blocks by 12 to 27%. The reason for the lower efficiency of 'strips' was due partly to the undesirable shape of the blocks. Even compact blocks with controls were less efficient, due to the fact that controls occupied more space and had that space been utilised for increasing the number of replications the magnitude of the error variance could have been reduced more.

The relative efficiency of various incomplete block layouts for the  $6' \times 2'$  plot size without and with recovery of inter-block information, as compared to simple randomized blocks, was as follows:—

148 *Symposium on Statistical Methods in Plant & Animal Breeding*

No. of progenies	Design	Relative efficiency (both series of simple randomized blocks = 100)	
		Without recovery	With recovery
64	Double lattice	85.0	100.6
	Triple	89.0	100.7
	Cub	96.6	100.0
	Symmetrical incomplete blocks	89.6	100.0
128	Double lattice	7.6	100.6
256	Double	100.1	111.6
	Triple	104.3	107.3
400	Double	100.4	103.6
Average		90.0	103.0

The results showed that for progenies upto 125 the designs were distinctly less efficient as compared to the simple randomized blocks, when the inter-block information was not recovered. For a larger number of progenies however, the efficiency was slightly higher than of simple randomized block layout. With the recovery of information the efficiency was the same as that of the simple randomized blocks for progenies upto 125 and increased slightly for larger number of progenies. So far as the present results were concerned the designs did not seem to be useful considering the complicated nature of the field arrangement and the labour involved in the statistical analysis.

Mr Sahasrabudhe added that in these designs the comparisons of the progeny means were done with unequal precisions as different errors had to be used for testing the significance of the differences when the progenies occurred in the same or different incomplete blocks, except in the case of symmetrical incomplete block designs, where due to the balanced nature of the design the progeny means could all be tested with equal precision. This latter design, however, required a certain minimum number of replicates,  $p + 1$ , if  $p$  progenies occurred in an incomplete block and could not be employed where the available seed was not sufficient for sowing the minimum number of replicates.

Dr L. A. Ramdas (Poona) referred to the relationship between agricultural meteorology and plant breeding and explained how drought resistance of different varieties could be tested in the laboratory by controlling humidity and temperature.

Mr R. D. Narain (Cawnpore) stated that there appeared to be a considerable similarity in the statistical approach to plant selection and to the

study of human abilities. He illustrated his remark by explaining how the discriminant function could be used in the evaluation of answer papers of examinations to assess intelligence.

Prof. S. Ranjan (Allahabad) felt that too much attention had been paid to plant yield in selection work in the past and too little to the nutritional value of improved varieties. He emphasised the need of physiological study in conjunction with plant breeding.

Dr. C. Chandrasekar (Calcutta) illustrated some applications of statistical methods to the study of human genetics. The science of human genetics presented greater difficulties than those presented by plant or animal genetics since neither ancestry and genotypes of human beings could be known with accuracy, nor could their matings be controlled. In such circumstances 'The principle of random mating' offered immense possibilities for the human geneticist. The principal assumptions of this method were that the matings took place at random and that the genotypes were uniformly distributed in the population. With the help of this method several useful results could be calculated. For instance, the calculation of the proportions of normal and affected progenies in case of characters controlled by dominant or recessive genes. Similarly, it could be used to predict occurrence of certain characters and the results could be compared with those actually observed. One such character was the ability to taste phenyl-thio-urea in small dilutions. Expectations worked out for this character for American population were well corroborated by observed data. However, observational data on the occurrence of albinism in England differed very considerably from expectation and thus failed to substantiate the assumptions. That gene frequencies may vary substantially even in groups living close together was well brought out by the distribution of blood group data recorded at Calcutta. Hence it appeared exceedingly difficult to obtain actual results rigidly satisfying the condition of even distribution of genotypes. For the selection of homogeneous populations, primary surveys of blood groups, response to phenyl-thio-urea and such other characters might offer useful guidance.

Prof. Madhava (Delhi) said that he was not a plant or animal breeder but that his contacts with Drs. Sukhatme and Panse had aroused his interest in the subject and that he would say a few words as to how the statistician got involved in problems of that kind. It was true that the geneticist had to deal with phenomena due to a number of causes. He must, therefore, have resort to statistical methods. The statistician had also to enlarge the domain of his activities because statistics was a unifying element in all

## 150 *Symposium on Statistical Methods in Plant & Animal Breeding*

sciences. However, he felt that there was too much emphasis placed on pure statistical methods. He had been reading in an amateurish way recently some of the books of Haldane and other authors. But there he noticed that his difficulty was not in following the general subject matter. He came across certain equations of the kind

$$U_n = U_n + K/(U_n + 1 - K) \\ = (U_n^2 + U_n)/(U_n + 1 - K)$$

He found that in Haldane's lectures there had been no formal solution of that equation. It had been solved in an approximate way only. The solution was valid where the generations did not overlap as in annual plants. He also had come across the transcendental difference equation in Fisher's book

$$U_n = Kc$$

Pure mathematics had lagged and had not been able to devote itself to the rigors of a complete solution of equations of this type. Furthermore, one comes across certain types of linear equations of the 22nd degree in the evolution of pedigree proportions. Obviously, the solution of an algebraic equation of this order is impossible except through refined calculating machines.

Mr V. G. Pendharkar (Bombay), in a written contribution, made the following observations on the Etah Goat Breeding Experiments in connection with the application of statistical methods to animal breeding.

Although the Goat Breeding Experiment carried on at Etah, to which Dr Sukhatme had referred, failed in its chief objective, viz., to breed a type of goats from the existing types with a higher milk-yielding capacity, considerable importance attached to the experiment on account of the revelation of the immense complexity of the problems which were inherent in such schemes.

A clear statement of the objective was the first necessity in any experimentation. The Etah Scheme aimed at producing goats with a higher milk yield. But the term higher milk yield could have several interpretations, such as a higher milk yield for the whole period of lactation, or a higher average milk yield for the period the animal is in milk, or again a higher average daily milk yield calculated on the basis of the interval between two kiddings. As the length of a lactation and therefore the length between two kiddings can to an extent be manipulated, it would appear desirable to aim at securing an increase in the average daily milk yield during the period the animal is in milk.

The selection of the raw material comes next. In order to have scope for improvement the raw material must possess a considerable amount of genetic variation in the values of the character with regard to which experiments are being made. The yield of milk, however, is a character notoriously susceptible to a variety of factors such as the time of the day, the stage to which a lactation has progressed, the order of the lactation, the type of feed given to the animal, the climate and so on. Appreciation of this fact implies (a) that in planning the experiment and during the analysis of the data due care has to be taken so as to prevent the mixing up of the effect of such factors and the effect of genetic selection and (b) that as a preliminary to breeding experiments a good deal of data should be collected and analysed so as to yield information regarding the relative importance to be attached to each of them. With a knowledge of the precise effects of the different extraneous factors on the character experimented upon we should be in a much favourable position for conducting the experiments. Besides, it is well known that the animal breeder attaches considerable importance to colour and configuration in fixing his 'pure' types from the animals. Whether such "pure" types chosen so far in this country do in fact correspond to a "pure" type for a quantitative character such as milk yield or the yield of meat, etc., in the sense that the genetic part of variation in the incidence of these characters is negligible is a point which remains to be proved. In the Etah experiment most of the Bahari goats were chosen from a certain part of the country which is supposed to have a "pure" herd. The results of the experiment are not conclusive as to whether the goats and bucks were homozygous with regard to milk yield or not but there is considerable ground for supposing that this might have been the case. Prior knowledge particularly regarding the correlations between parents and offsprings would have been of immense help to the planners of the experiment had it been available. Collection and analysis of data regarding the incidence of such characters in the so called "pure" types seems an urgent prerequisite for the animal breeding experiments in this country.

Secondly, information regarding the incidence of the character in one part of the raw material, namely, sires, is lacking. We can only estimate a sire's worth from the performance of his daughters which is too late for our purposes. Nothing can be more disheartening than to find after spending several years and considerable amount of money that (a) either the sires were probably homozygous with the dams and therefore there was no scope for improvement or (b) that they transmitted a low milk-yielding factor and actually spoil the herd. Proper selection of the sires is therefore a very vital factor for the success of the experiment.

We must have a method of discriminating between good and bad sires and that too preferably at the start of the experiment or at any rate in its early stages, say, in the course of the first or second lactation of their progenies. This necessitates a very careful planning of the experiment and thorough analysis of the data by modern advanced statistical methods at each stage.

The necessity of such discrimination becomes all the more paramount on account of the practice of having a relatively small number of sires as compared to the dams. One of the main grounds of criticism against the Etah experiment is that the number of bucks was very small to start with and in the later stages the male progeny of only one of these bucks (48 M) was given great prominence. Since nothing was known regarding the ability of the original sires and dams and the first progeny for some unaccountable reason gave a higher milk yield than their dams, the experimenters were lured into a false sense of security. By the time the true worth of the sires began to show itself the experiment was too far advanced. The lesson to be learnt is to have a relatively larger number of sires at the start of the experiment say one sire for every 15 dams or so. As far as possible the sires should be selected with reference to their mothers' lactation performances. All possible data regarding the performances of their relatives should be collected and studied. In the absence of herd books exact quantitative information would be impossible to obtain. And in any case the information will have to be supplemented by direct evidence obtained from the performance of their progenies. For this purpose the sires for the different groups should be interchanged for the different kiddings according to a pre arranged plan so as to compare their abilities after allowing for the difference in the milk yield of the progeny caused by the variation in the dams. Even for such a scheme it would take 8-10 years before the true worth of the sires can be assessed. Though this may be so, the need for carrying out an experiment on the above lines can hardly be doubted. It may be possible to devise short cuts to forecast the sire's ability based on a study of material collected for a short period.

As mentioned above the first progeny of the goats on the Etah farm gave higher milk yield than their mothers. Various causes such as difference in the orders of lactations of mothers and daughters, difference due to the early nomadic life of the mothers, *i.e.*, the foundation stock and the farm life of the daughters, hybrid vigour, etc., have been adduced for this phenomenon. Whatever may be the actual cause the phenomenon is worth investigating further because of the important issues it raises with regard to the breeding plan. Perhaps the easiest way of overcoming the difficulties created by such a phenomenon would be to regard the first progeny and

not the original animals as the foundation stock in the absence of information regarding the previous performances of the original animals. This would certainly mean some expenditure and loss of time but we would be on a surer ground in as much as we would be eliminating possible differences in milk yield caused by environmental factors. Secondly we would have complete data for progenywise comparisons.

Associated with any such scheme must be an efficient system of recording and regularly analysing the data collected. Decisions regarding mating, culling, etc., should only be based on the results of the statistical analyses carried out by appropriate methods. We can then be assured of a reasonable chance of success.

Mr A. R. Sen (Lucknow) in a written contribution made a survey of available statistical methods for the detection and estimation of linkage in genetics. He dealt with the method of maximum likelihood for estimation of linkage, alternative procedures when single factor segregations were disturbed and with the planning of efficient experiments for linkage studies.

Mr K. Ramiah (Cuttack) in winding up the proceedings said that they had been listening to a series of important and thought-provoking papers on various aspects of statistics in its application to plant and animal breeding. He wished that many others who were actually engaged in plant breeding research were present at the meeting. The whole point of the discussion was that plant and animal breeders did not take enough help from statistics. As for himself he did not see any reason why the breeder should fight shy of statistics. After all statistics was only a tool and it was necessary for the breeder to utilise this tool to make sure that he was proceeding on right lines.

He did not entirely subscribe to the accusation that breeders did not utilise statistics and said that statistics as a science in its application to agriculture and field experiments was hardly three decades old and some of the problems dealt with by the previous speakers have been under investigation only within the last few years. He said he was one of those lucky few to be associated with men who were just bringing the idea of probable error and duplicate plots in agricultural experiments in India nearly thirty years back. Great improvements had taken place since then. He felt that in using statistics the availability of trained staff and other facilities had to be taken into account. Unfortunately, the statisticians were suggesting designs for the breeders without a correct idea of their requirements and the facilities available on the spot. The breeders were also, with a blind enthusiasm for the latest methods, trying to adopt them without understanding the

## 154 *Symposium on Statistical Methods in Plant & Animal Breeding*

limitations and difficulties involved. He was glad to find from Mr Sahasrabudhe's paper that a simple randomized blocks layout was really more efficient in several cases than some of the later improvements like the incomplete block designs. While it was necessary to look for efficiency in a design, the practical convenience was, he considered, much more important particularly in out-of-the-way research centres with limited staff and inadequate facilities. Mr Ramiah requested the statisticians particularly to look very carefully into the plant breeding problems before they gave advice. He wanted to warn the statisticians that they should not rest content with giving advice and examining the data collected but should also look into how the data had been collected.

He said that Indore had been unique in respect of an intimate association between the statistician and the breeder but this advantage did not exist elsewhere in India. He was glad Dr Panse had shown them how statistics could be of help in plant breeding and had drawn their attention to the value of the discriminant function in plant selection, but considering that very few statisticians had taken up the subject seriously, he considered that the progress made in India in the use of statistics in plant breeding was creditable. He hoped that Dr Panse's results on cotton would soon be tested out in other crops and he was looking forward to their use in rice breeding. Referring to his recent visit to Denmark and Sweden, two of the most advanced countries in agriculture in Europe, he said he was surprised to see that the Fisherian technique was not adopted in field experiments in those countries. The workers there were content with the classical systematic arrangements in field trials on the plea that the application of randomization entailed practical difficulties and they thought that their method of balancing the varieties within each block gave a better scope for an easy visual comparison. The impression he got from a perusal of the recent Imperial Bureau publication on Soviet Genetics was that they were denouncing their faith in modern genetics, the basic science for plant and animal breeding, and were going back to Burbank's methods!

With regard to animal breeding the position in India was very much worse than in plant breeding as far as the use of statistical methods was concerned. This was entirely due to the fact that most of the animal breeders in the country were not acquainted with statistical methods, but there were signs of an awakening in recent years. Until recently there was not even unanimity of opinion among experts with regard to the parents to be used for crossing. Now that young men are being sent abroad for training in animal breeding and statistics he hoped that a better chapter would be written about animal breeding in the near future.

# SPECIFICITY OF BACTERIAL SYMBIOSIS IN APHROPHORINÆ

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**SCALE INSECTS** when they contain symbiotic yeast-like micro-organisms, show specificity of symbiosis. The technique which gives such results is very simple. Smears of tissue containing the symbiotes are carefully illustrated with the help of a camera lucida and the drawings selected to show not merely representative forms but also to give a summary picture of the range of polymorphism observed. Such an illustration would represent all common polymorphic forms of the germ. If properly undertaken each picture would be different from others and exhibit specificity of symbiosis among insects as has been shown to be the case in some scale-insects<sup>1</sup>. This method was an extension of a plan<sup>2</sup> to classify commercial lac insects by a microbiological method and a subsequent study<sup>3</sup> has shown that lac insects also harbour specific symbiotes.

The method was extended and some interesting results were obtained. Morphologically the genuine lac insects and pseudolac insects were once classed in the same genus by an expert coccidologist like Green<sup>4</sup>. Taking three genera of lac insects, *Lakshadia*, *Metatarchardia* and *Tachardina*, their symbiotes show a corresponding generic difference. *Lakshadia* have yeast-like symbiotes, *Metatarchardia* contains an actinomycete,<sup>5</sup> while *Tachardina* species have bacteria. Now there is an insect, *Tachardina silvestrii*, which has not yet been recognised as an independent species by systematists. A smear from this insect easily distinguishes it from other sister species,<sup>6</sup> thus indicating the value of the new method when it can be applicable.

*Tachardina lobata* has two favourite host plants, *Michelia champaca* and *Guazuma tomentosa*. Much to my surprise I found there are two separate insects<sup>7</sup> which do not differ morphologically but are separate with regard to host-selection and also with regard to the symbiotes they harbour.

Just as pseudolac insects could be separated as a class from lac-insects proper, by generic differences in their respective symbiotes, some insects producing wax and pseudowax have been likewise separated. *Cerococcus* species secrete wax and they harbour yeast-like symbiotes, while a new genus

has been created *Coricoccus* for insects<sup>4</sup> that do not produce wax proper and contain bacteria in symbiosis<sup>5</sup>

It has been previously emphasized that morphologists do not usually consider physiological characters<sup>2</sup> If these be given due consideration specificity of symbiosis would also become a part of the physiological system of an organism which would be an additional aid in classifying the hosts exhibiting it For example it is being shown in this communication that two species of *Aphrophorinae* differ in colour, a fact not fully exploited and further that their symbiotes also differ and it is these germs that produce their respective pigments To admit that the colour of these insects differs from species to species is to imply that their producers, the symbiotic germs, also differ and independently this is being established in a series of studies which began with that of *Cicadella viridis*<sup>10</sup> Specificity of symbiosis as far as yeast like symbiotes are concerned is relatively easy to establish, the method is not easily applicable to bacteria on account of their small size and further because the germs seem to disappear from the bacteriomes<sup>11</sup> when the adults are about to lay the eggs The method has more than one limitation but whenever the technique is applicable it is as reliable as any morphological method When colour is recognised as an important character for differentiating species I do not see how the germs that produce these pigments can be overlooked Since this point had not been recognised there appeared a great gap between the papers that established specificity of symbiosis where the symbiotes were yeasts and the series of communications that are being offered<sup>10</sup> to prove that there is a similar specificity of symbiosis even when the symbiotes are bacteria

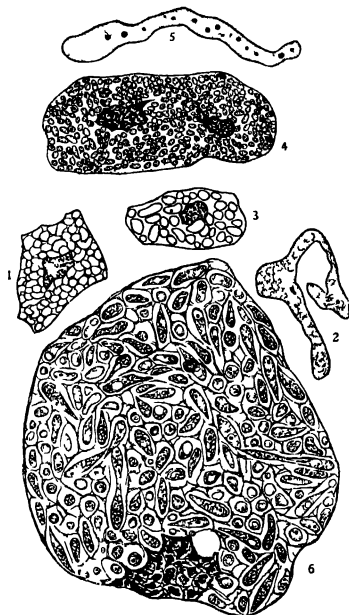
Every systematist recognises that it is easier to separate genera than to establish different species The difficulty arises when morphological differences are small It is precisely in such cases that physiological differences are greater For example, *Tachardina silvestris* and *T. lobata* have different host plants and different bacteria and also different colours Dogmatic systematists have not recognised such facts as certainly Chamberlin<sup>12</sup> has not done while my subsequent work has justified the new standpoint

I shall begin with symbiosis in *Aphrophorinae* of which only two species are commonly found in Europe Haupt's monograph<sup>13</sup> of Homoptera gives *Aphrophora salicina* Goeze, also named *A. salicis* De G. as 9-11 mm long and relatively less distributed *Aphrophora alni* Fall is 8-11 mm long and is found all over Europe and even in north China *A. spumaria* L.<sup>14</sup> is its another synonym and perhaps for this reason there appears to have been some confusion in identifying the insect At any rate *A. alni* (*A. spumaria*)

is certainly the more easy to find and has consequently been the first species to be investigated. A popular book on natural history like that of Lydekker<sup>15</sup> mentions only *A. spinaria* as a representative of the class of spittle insects. Sulc<sup>16</sup> records it in his classical communication on symbiosis in 1910 stating: "Bei *A. alni* eine ganze Menge von eigenartigen grossen Bakterien, die im besonderen Zellen aufgespeichert neben der Hefe ganz gut im Organismus prosperiert" (p. 36). He thus records a yeast and a bacterium in *A. alni*.

In 1912 Buchner<sup>17</sup> published a monograph on symbiosis incorporating studies on a species of Aphrophora. In the text he does not refer to Sulc's finding just mentioned. He illustrates a larva which bears no designation in the explanation to the plate where the coloured figure is given. Unfortunately I was led to state that Buchner did not know the species.<sup>11</sup> Buchner in his treatise on p. 72 states that the species under question is *A. salicis*, the rarer insect. He however shows on Plate 11, Fig. 6 large bacteria like cell inclusions which to quote Sulc appear peculiarly large ones. The yeasts to which Sulc refers are also illustrated by Buchner on Plate 11 in Fig. 5. The illustrations fully confirm Sulc's previous record and added to this is the probability that Buchner also handled an insect which was by far the more common, the investigated *A. alni* and not *A. salicis* as he states. In his second monograph<sup>18</sup> which appeared in 1925 Buchner further investigated the same insect where he confirms his previous findings of 1912 with the addition of a third yeast like symbiote. Strange enough he does not correct the discrepancies to which attention has been drawn here.

*A. salicis* was actually first investigated by Buchner<sup>14</sup> in 1925. On p. 208 he classes the insect as a disymbiotic host. One symbiote is a long bacillus typical of its class to which no bacteriologist would object. He illustrates these germs in Fig. 4b on p. 103. The other symbiote is supposed to be a fungus or yeast belonging to the mysterious genus *Cicadomyces* and the species under question has been named by Sulc as *Cicadomyces aphrophorae salicis* and quoted by Buchner<sup>17</sup> on p. 102 under the impression that he was studying *A. salicis*. It should have been *C. aphrophorae alni*. A recent book on insect microbiology<sup>19</sup> by Scheinhaus also does not point out this mistake. The fact that so far such an obvious error has not been discovered is to be interpreted as due to the supposed germ having been very poorly described or according to me for not having existed at all. A tissue cell containing these *Cicadomyces aphrophorae salicis* shown by Buchner<sup>14</sup> in his Fig. 7 on p. 107 is reproduced here as Fig. 1. In a section



FIGS 1-6 1-5. 1 Supposed symbiote of *A. salicis* as an intercellular micro organism from Buchner Fig 2 Supposed symbiote of *A. salicis* isolated—After Buchner Fig 3 Supposed symbiote of *A. alni* as infection form entering in egg—from Buchner Fig 4 Supposed symbiote of *A. alni* in a tumour cell of the adult insect—from Buchner Fig 5 Supposed symbiote of *A. alni* isolated from the tumour of an adult insect—from Buchner Fig 6 Picture of genuine yeast like symbiotes from a Chick—from Buchner

these bodies do superficially resemble yeasts. But once they are isolated from the tumour these germs appear to have bizarre forms which I have been also illustrated by Buchner in his Fig 4a p 103. One of these bodies is reproduced here as Fig 2. The object looks any thing but an yeast. These Cicadomyces have one appearance in sections and quite another when seen isolated. This is certainly nothing to do with polymorphism.

While discussing symbiosis in *Cicadella viridis*<sup>10</sup> I have mentioned that Cicadomyces are protoplasmic debris. In the tissue cell they lie in large pieces such as Fig 2 here illustrates intertwined and folded so that none can be cut longitudinally. Imagine a ball of cord or thick rope being cut across the cords in cross section would always appear as round objects. Such is the case with Cicadomyces or elongated pieces of protoplasm which are always seen cut across and thus as round objects giving a uniform picture in practically all homopterous insects. The more these objects are studied the more is the uniformity discovered and it becomes impossible to give a specific description of any of these so called germs. With Gimenez stain they take the characteristic blue plasma stain. They show no organized nucleus but now and again only chromatin residues. Vacuoles are usually absent while they are always present in yeasts and in fungus mycelium. Yeasts and bacteria have some kind of membrane which enables them to withstand disintegration and even digestion with pepsin in dilute hydrochloric acid. The Cicadomyces do not possess any such membrane and are easily digested and disintegrated. None of them has been cultured so far while no microbiologist has seen anything like them anywhere outside the field investigated by workers on insect symbiosis. I must again quote from Gregson<sup>12</sup> "During recent years much has been published regarding various intracellular symbiont like bodies. In several instances it has proved difficult to classify these bodies biologically and in a few cases it has not even been established that they are living units. This is perfectly true of Cicadomyces<sup>10</sup>. It therefore means that *A. salicis* contains only one symbiote the bacillus first observed by Buchner.

In 1912 Buchner confirming Sulz's findings illustrated two symbiotes of *A. alni* although he had mistaken it for *A. salicis*. Of these two symbiotes one was a giant form of bacterium shown in his Fig 6 Plate 11 and the other an yeast or rather *Cicadomyces aphrophora alni* Sulz which was illustrated in Fig 5 Plate 11. In 1925 Buchner reinvestigated *A. alni* now correctly identifying the insect. The previous findings naturally would have been of greater value if Buchner had also corrected the minor but obvious mistake of not having properly identified the species in 1912. The memoir

of 1925 records in all three symbiotes in *A. alni* for which Buchner uses the synonym *A. spumaria* and under this name classes it as trisymbiotic insect on p. 210. The three symbiotes are illustrated on p. 113 with their respective tumour cells facing them for easy comparison on p. 112. The additional symbiote is a rather yeast-like object evidently another *Cicadomyces*.

The largest symbiote in *A. alni* is Sulc's *Cicadomyces a. alni*. In Fig. 11a Buchner illustrates it as seen in a histological section where the tissue cells contain yeast-like or round objects ready to pass into an egg. One such tissue cell full of *Cicadomyces* is reproduced as Fig. 3 here. I am not sure if the author means to show two species of these mysterious germs or only one. However *Cicadomyces a. alni* is shown by Buchner in a section of the adult insect in his Fig. 9a p. 112 which is copied here as Fig. 4. The supposed germ when seen isolated appears in Buchner's Fig. 10a p. 113 with the same fantastic forms as were mentioned in connection with *A. salicis*. One of these forms representing *Cicadomyces a. alni* shown isolated is reproduced here as Fig. 5. *Cicadomyces a. alni* Fig. 5 compares very well with *Cicadomyces a. salicis* Fig. 2 so much so that neither in illustrations nor in living condition can there be any difference between them. Steinhaus<sup>16</sup> who apparently believes in their existence remarks in connection with *Cicadomyces a. alni* Sulc that this species is without an adequate description. If a picture be drawn to show the polymorphism exhibited by the chief symbiote of *A. alni* Fig. 5 here with that of *A. salicis* Fig. 2 here there would hardly appear any difference. This is to be expected if both are protoplasmic debris. All the remarks passed previously with regard to Sulc's *Cicadomyces a. salicis* apply with equal force here.

In 1910 Sulc<sup>18</sup> mentioned two symbiotes: one is *Cicadomyces a. alni* just dealt with and the second is supposed to be an exceptionally large bacterium. Buchner<sup>17</sup> illustrated these bacteria of Sulc in his thesis of 1912 in Fig. 6 Plate 11 and in his monograph<sup>14</sup> of 1925 on p. 113 in Fig. 10c. These bodies are so large that no bacterium can be compared with them. Just as *Cicadomyces* occupy an anomalous position among yeasts this bacterium of Sulc does among bacteria. Although Sulc did record finding a bacterium in *A. alni* Buchner has not mentioned Sulc's finding either in his work of 1912 or in that of 1925. His Fig. 10a offered in 1925 remains unidentified either as bacterium or as fungus and my interpretation is that it is neither. Buchner merely calls it a symbiote.

Such long cell inclusions as these bacteria-like bodies appear have not been met with in any other insect and as such struck to me as specific.

I could not have spent more time to study these bodies critically. They stain blue with Giemsa as typical protoplasmic pieces, are very delicate and do not stand disintegration, lack nucleus and many do not have vacuoles. I have done my best to cultivate them without any success. I am convinced they are not living entities but pathological products of protoplasmic origin. Objects of the same shape but shorter in length are met with in many insects.

The third symbiote which has been discovered by Buchner in 1925 and not mentioned in his thesis of 1912 is apparently a species of *Cicadomyces*, so that I am the more convinced that this new symbiote is only a stage of protoplasmic disintegration comparable with the main *Cicadomyces*. Not being different in kind to the other *Cicadomyces* further criticism is not necessary.

To show what a picture genuine yeast-like symbiotes offer I have copied from Buchner<sup>17</sup> and reproduced his illustration Fig 4 Plate 6 as Fig 6 here. They show yeasts in the fatty tissue of a Cicad, some yeast cells are seen budding which is hardly met with in sections showing *Cicadomyces*. The germs are separated from one another by residues of protoplasm from the tissue cell. Vacuoles are seen and likewise nuclei. Fig 6 representing real germs offers a great contrast to Figs 1 and 4 and 5 which all contain *Cicadomyces* or protoplasmic debris. My object has been to study the real symbiotes to culture them and to see their role *in vitro*. The study of *Cicadomyces* has taken me to pathological histology which I confess has been rather forced upon me. I have also attacked this problem with respect to my predecessors who have been pioneers in the field but we are all liable to error and I have criticised them after much labour and patience in convincing myself.

#### SUMMARY

*Aphrophora alni* and *A. salicis* each have one bacterium in symbiosis. In smears they are specifically different. *A. salicis* has a long bacillus, *A. alni* a short and delicate bacterium. These germs produce the pigments of their host insects: the symbiote of *A. salicis* an ochre yellow pigment like the colour of the insect; that of *A. alni* red-brown which is the colour of this species. Morphological and physiological tests have shown that the isolation of the symbiotes has been correct.

Sulc and Buchner have illustrated mysterious yeasts or fungi in symbiosis with these insects. These supposed germs are placed in a new genus *Cicadomyces*. Details are given to show how they do not represent living entities. Even the authors themselves have subsequently discarded their earlier nomenclature and have designated these bodies simply as symbiotes.

feeling themselves doubtful regarding the real nature of the objects they have illustrated. These are best interpreted as protoplasmic debris or pathological products without any nucleus, but merely with chromatinous residues, without any membrane to resist disintegration and digestion and above all incapable of being cultivated and indicating any evidence with regard to their function. These Cicadomyces show a great contrast to real yeast-like symbiotes which have been also illustrated for comparison.

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## THE MODE OF ACTION OF NERVES ON UNSTRIATED MUSCLE

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THE views regarding the mode of action of nerves at their endings are well known. In the present research an attempt has been made to determine the mode of action of nerves on unstriated muscle. Singh (1938 a) has shown that unstriated muscle shows two kinds of contractions. One kind is produced by alternating current and spontaneous activity (Singh, 1939), and the other by addition of substances to smooth muscle from without. If nerves act by producing a chemical substance outside the muscle fibres, then the resulting contraction would be of the second kind. Narayana and Singh (1944) found that in the dog's stomach the calcium required for contractions produced by acetylcholine or by vagus stimulation was more ( $0.005-0.01 M CaCl_2$ ) than that required for the contraction produced by alternating current ( $0.002 M CaCl_2$ ). The significance of this finding was not then realised, later on it was found to be a failure when the contraction belonged to the potassium group (Singh, 1945) thus suggesting that the contraction of the dog's stomach produced by stimulation of the vagus nerve was due to the secretion of a substance outside the muscle fibres, presumably acetylcholine.

### EXPERIMENTAL

The muscle used in these experiments was from the stomach of the frog *Rana tigrina*. Two kinds of muscle nerve preparations were made. In one, the entire stomach tied at the two ends, was suspended in a bath and the mesentery placed on a pair of electrodes, this recorded the contractions of longitudinal fibres. In the other, the two nerves supplying the anterior and posterior surfaces of the stomach were dissected down to a common segment which was then cut out transversely and then bisected longitudinally at the greater curvature, the mucous membrane was subsequently removed. This provides an ideal nerve-smooth muscle preparation for recording the contractions of the powerful circular muscle fibres. During stimulation of the nerves, the solution was lowered in the chamber, and the preparation suspended in the air. The nerves were stimulated by maximal induction shocks for 30 seconds every 15 or 20 minutes. As the responses of frog's

stomach are so variable, three pieces were taken from another or the same frog and the responses to alternating current, potassium and acetylcholine (1 in 5000-2500) were obtained for comparison. This high concentration of acetylcholine was used as the frog's stomach is relatively insensitive, though responses obtained by nervous stimulation are usually powerful, inexcitability to nervous stimulation has not yet been found. During the same season the responses from the same batch of frogs are similar. The contractions by all kinds of stimulation are quite regular and can be obtained for several hours.

### RESULTS

The contractions produced by the longitudinal fibres are feeble, while those produced by the circular fibres are powerful when stimulated through the nerves, indeed the contractions are as powerful as those produced by any other form of stimulation. Spontaneous contractions may interfere, but often they are quite small. The latent period of the contraction produced by nervous stimulation is 5-20 seconds, adaptation may be rapid so that the response may begin to decline before the period of stimulation is over, or it may continue to increase for about 15 to 20 seconds after stoppage of the stimulus. The response is a twitch, but sometimes the relaxation is slow.

Nervous stimulation of circular fibres produces a contraction similar to that produced by alternating current and spontaneous activity while stimulation of longitudinal fibres produces a contraction which is similar to that produced by potassium and acetylcholine.

### *Action of Drugs*

*Effect of atropine* — 1 in  $10^7$ - $10^8$  atropine sulphate inhibits the response to potassium, acetylcholine and of longitudinal muscle to nervous stimulation. 1 in  $10^8$  then improves the response to nervous stimulation and potassium. The response to alternating current may be similarly affected, but usually 1 in  $10^7$  improves the response to alternating current (Singh and Mrs. Singh, 1946), as well as the response to nervous stimulation of circular fibres. Higher concentrations are depressant to both. 1 in  $10^4$  is depressant to all forms of stimulation (Fig. 1).

Acetylcholine action is sometimes very susceptible to the inhibitory action of atropine, 1 in  $10^7$  and higher concentrations of atropine completely abolishing the response produced by 1 in 5000-2500 acetylcholine. At other times the muscle is very resistant, and the response persists even with 1 in  $10^4$  atropine. When the response is a tonic contraction, then it is very

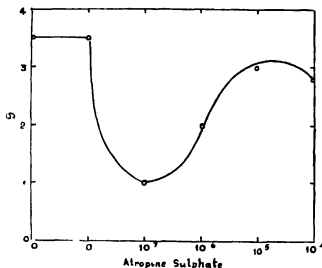


FIG 1 Frog stomach, action of atropine on the response to nervous stimulation of longitudinal fibres

susceptible, when it is a series of twitches, then it is more resistant. Calcium converts the tonic contractions of unstriated muscle into twitches (Singh, 1938 *b*). It would therefore appear that the membranes of muscles which respond by twitches contain more calcium and hence are less permeable than those which respond by tonic contraction. *Mytilus* muscles, which give twitches, swell less in various solutions and so are less permeable than others (Singh, 1938 *c*, 1944 *a*). This is in agreement with Dale's view that the chemical transmitter in the case of vagal stimulation is secreted at a place at which atropine cannot penetrate. This is best explained if it is assumed that the chemical transmitter is liberated in the outer zone (Singh, 1944 *b*). The twitches produced by acetylcholine may be even increased by atropine (1 in  $10^6$ ).

*Effect of eserine*—Previously it was found that small concentrations of eserine sulphate have an inhibitory effect on the response to acetylcholine (Singh, 1939). This was confirmed in the present series of investigations. It was found that tonic contraction is very susceptible to inhibition, 1 in  $10^7$  completely abolishing the response (Fig 2). Thus with eserine also tonic contraction is more susceptible than twitches. The response of longitudinal muscle to nervous stimulation is affected similarly, 1 in  $10^7$  causing inhibition, and 1 in  $10^6$  and 1 in  $10^5$ , causing increase, the response being more resistant than that produced by acetylcholine. The response to alternating current and nervous stimulation of circular muscle is increased by 1 in

$10^{-7}$ - $10^{-6}$ , and depressed by 1 in  $10^6$ . The response to potassium may be affected either way. The response to nervous stimulation of circular fibres may be affected also as in the case of longitudinal fibres.

The response to nervous stimulation is characterised by long latent period, and by the fact that it may continue long after cessation of the stimulus. The question arises whether it is a composite or a single response. In one experiment only it was found that, to begin with, there was a single response, as the concentration of eserine was increased from 1 in  $10^7$  to 1 in  $10^6$  the response was gradually split into two, one occurring during and the other on cessation of stimulation. The one occurring during stimulation was suppressed and the other occurring on cessation was augmented by 1 in  $10^6$ - $10^4$  eserine, the former resembling the response produced on stimulation of circular fibres by nerves. Thus nerves produce responses during and after cessation of stimulation, and these responses are affected differently by eserine. Other forms of stimulation also produce similar responses (Singh 1938 a, 1939, 1942).

*Effect of a brucine* — 1 in 10 improves the response to alternating current, and to nervous stimulation of circular fibres, or this action may be produced by smaller concentrations if 1 in  $10^7$  is inhibitory. Higher concentrations are depressant. 1 in  $10^7$  depresses the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres. 1 in  $10^6$  has also a depressant action. 1 in 10 may potentiate the response to potassium as in *Mutilus* muscle (Singh, 1938 a) and nervous stimulation. Here again the tonic contraction by acetylcholine is more susceptible than twitches, 1 in  $10^7$  completely abolishing the response, and if 1 in  $10^6$  is depressant, then the response to nervous stimulation is more resistant, as with atropine and eserine.

*Effect of acetylcholine* — The action of acetylcholine resembles that of eserine. It has two kinds of effects. First, it has an inhibitory action on the response to alternating current, potassium, acetylcholine and nervous stimulation of longitudinal fibres in small concentrations (1 in  $10^6$ - $10^7$ ), in larger concentrations (1 in  $10^5$ ), it has a potentiating effect. Secondly, it has a potentiating action on the response to alternating current and nervous stimulation of circular fibres in small concentrations, and an opposite action in larger ones.

The question arises, why, if the response to nervous stimulation is due to liberation of acetylcholine, the presence of acetylcholine in the saline then should not enhance the action of the former. The depressant action of acetylcholine is due to contracture or adaptation. In frog's stomach

contracture does not occur with small doses ( $1$  in  $10^5$ ) as it is relatively insensitive, and adaptation to chemical stimulation is slow. It is thus found experimentally that  $1$  in  $10^5$  acetylcholine potentiates the response to nervous stimulation.

#### *Action of Divalent Cations*

*Effect of calcium*—The optimum concentration of calcium for the response to alternating current is  $0.0028$ – $0.0042$   $M$   $CaCl_2$ , and for acetylcholine  $0.0028$   $M$   $CaCl_2$ , for potassium it is  $0.0014$   $M$   $CaCl_2$  and for nervous stimulation  $0.0014$ – $0.0028$   $M$   $CaCl_2$ . In the absence of calcium, or if the concentration of calcium is reduced, there is a temporary state of hyper-excitability to potassium, acetylcholine and nervous stimulation.

Excess of calcium is depressant, but excitability again increases in about  $0.007$   $M$   $CaCl_2$  as in avian and rabbit's gut muscle. The potentiating action of calcium to potassium as found in mammalian muscle is not found in frog's muscle, though this action is produced by excess of strontium.

*Effect of strontium*—Strontium increases the response to all forms of stimulation in concentration of  $0.0014$ – $0.0028$   $M$   $SrCl_2$ . The action of calcium on mammalian muscle is similar to that of strontium on frog's muscle. In frog's muscle strontium can replace calcium, as a matter of fact it may have a stronger effect. The stronger action of strontium appears to be due to diminished adaptation. This is probably due to diminished ionisation of calcium (Singh, 1944 c).

*Effect of barium*—Barium has two kinds of action, one resembling that of calcium and the other that of potassium in causing contracture. Owing to its latter action, it is usually depressant. The calcium effect can be shown by the fact that it can produce persistent contracture in the absence of the  $Ca$  ion, and shows the calcium effect on the response to potassium, if contracture is not caused. The calcium effect is more evident in heart muscle, this is probably because the calcium in it is more mobile thus reducing its sensitivity to chemical stimulation (Singh, 1946).

*Effect of magnesium*—Small concentrations of magnesium ( $0.0014$   $M$   $MgCl_2$ ) increase the excitability to alternating current nervous stimulation, potassium and acetylcholine. The response to potassium can withstand larger concentrations ( $0.0028$   $M$ ), as in *Mytilus* muscle. The favourable action of magnesium is probably due to de-ionisation of calcium.

#### *Action of Monovalent Cations*

*Effect of hydrogen ions*—The optimum pH for excitability depends on the buffer used. In borate it is about  $9.8$ – $5$  and in phosphate,  $8.7$ – $5$ . In

phosphate the excitability remains unaffected while in borate the response to nervous stimulation is likely to fail. As the pH is decreased to pH 7 the response decreases and thereafter the response increases up to pH 6.5. If the pH is further decreased the response to potassium, acetylcholine and nervous stimulation of longitudinal fibres increases up to pH 5.4-5.2 and the response to alternating current and nervous stimulation of circular fibres decreases. This is an important point in support of the view that response to nervous stimulation is due to the secretion of a chemical substance and resembles the potentiating action of hydrogen ions on the response to potassium in mammalian muscle. Gessel and his associates (1944) have presented evidence that acetylcholine liberated by vagal stimulation may be potentiated by acids which retard the breakdown of acetylcholine by choline esterase. As acids also potentiate the response to potassium it appears that this potentiation is not due to an action on cholinesterase only.

*Effect of lithium* The antagonism between the response to nervous stimulation of longitudinal fibres and that to alternating current is well shown by the action of lithium (Fig. 3). Lithium up to 0.04 M LiCl decreases the response to nervous stimulation of longitudinal fibres, acetylcholine and potassium, the responses to alternating current being increased. With

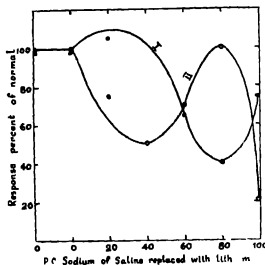


FIG. 3. Frog stomach. Action of lithium on the response to alternating current (Curve 1) and nervous stimulation of longitudinal fibres (Curve 2).

further increase of concentration of lithium, 0.08 M LiCl, the response to the former three is increased and to the latter decreased. With complete replacement of sodium of the saline with lithium, the response to all forms

of stimulation is decreased, though that to alternating current may increase, the response to nervous stimulation of circular fibres is similar to that to alternating current

*Effect of sodium*—Replacement of 20% of the sodium chloride of the saline by sucrose decreases the response to alternating current and nervous stimulation of circular fibres and increases that to potassium, acetylcholine and nervous stimulation of longitudinal fibres. The action is however variable and may be just the opposite. In electrolyte free medium (Singh, 1944 *d*), the response to nervous stimulation lasts for about an hour.

*Effect of ammonium*—The replacement of about 60 to 80% of the sodium chloride of the saline with ammonium increases the response to acetylcholine and potassium but decreases that to alternating current, nervous stimulation of longitudinal as well as circular fibres. In these muscles, ammonium may cause contraction.

*Effect of potassium*—The optimum concentration of potassium for the response to alternating current, nervous stimulation of circular fibres, acetylcholine and potassium is 0.01 *M* KCl, that for nervous stimulation of longitudinal fibres is one half to one-third of the above. Potassium and ammonium are depressant to nerve. A higher concentration of potassium would be antagonistic to leakage of potassium from the fibres.

#### *Action of Anions*

*Effect of bromide*—Low concentration, 0.02 *M*, may inhibit or increase the excitability, higher concentration, 0.04–0.06 *M*, has an inhibitory action. This action of bromide is probably related to its inhibitory action in the central nervous system. Higher concentrations, 0.08 *M*, potentiate the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres, the response to alternating current and nervous stimulation of circular fibres is depressed. The replacement of all the chloride by bromide is depressant.

The action of nitrate, iodide and thiocyanate is similar, but is obtainable with smaller variable concentrations, they are much more depressant.

*Effect of cyanide*—Small concentrations, 1 in  $10^2$ , are inhibitory, 1 in  $10^3$  may then increase the excitability. 1 in  $10^4$  may increase the response to potassium and acetylcholine and depress others. The concentration of anions required to produce the above effects varies. 1 in  $10^5$  may potentiate the response to nervous stimulation of longitudinal fibres, but usually cyanide depresses the response to nervous stimulation.

*Effect of Osmotic Pressure*

The effect of increasing the osmotic pressure of the saline is variable. Increase of osmotic pressure of the saline by adding sucrose to 1.4-2 times normal may increase the excitability to all forms of stimulation, at other times it may cause decrease. These variable results are probably due to the fact that increase in the concentration of potassium inside the fibres may antagonise either the excitatory or inhibitory action of ions outside the fibres, it will decrease the excitatory action of substances in the former case and increase in the latter.

*Effect of Temperature*

The optimum temperature for response to potassium and nervous stimulation of longitudinal fibres is 20°C. This suggests that in the case of latter stimulation the stimulating ion is the potassium. The optimum temperature for nervous stimulation of circular fibres is 20-25°C, that for acetylcholine, 30°C and for alternating current 20-25°C. The optimum temperature may vary with that of the saline, thus exhibiting adaptation (Fig. 4).

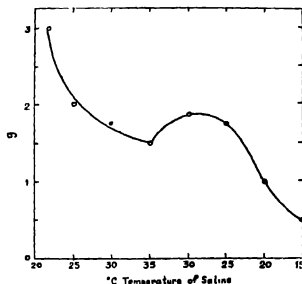


FIG. 4. Frog stomach. Effect of temperature on the response to nervous stimulation of circular fibres, note adaptation.

**DISCUSSION**

Dale and Feldberg have shown that acetylcholine is liberated from the stomach during vagal stimulation. This liberation of acetylcholine is of

functional importance as the contraction produced by vagal stimulation is similar to that produced by acetylcholine (Narayana and Singh 1944). During nervous stimulation of frog's muscle the contraction produced resembles that produced by potassium or acetylcholine. It thus appears that acetylcholine is liberated though the stimulating ion may be potassium. The potassium probably comes from within the cells as increase in potassium concentration in the saline does not augment the response. Acetylcholine probably causes depolarisation of the membrane increasing its permeability and causing leakage of potassium ions from within the fibres. These then cause stimulation as has been explained in connection with the occurrence of contraction caused by alternating current.

The action of atropine suggests that acetylcholine is liberated not around the fibres but in the adjacent zone (Singh 1944 b).

The occurrence of a contraction which is similar to that produced by alternating current when nerves are stimulated suggests the possibility of electrical transmission which will precede chemical transmission as found by Lorent de No in the central nervous system (McDowell 1944). The function of chemical transmission would be to impart tonic properties to the phasic contraction produced by electrical transmission. The function of cholinesterase in certain situations may be to prevent this action where it is not desired. Esterine by inhibiting its action would bring out the tonic function. It is possible that in some places in the body the transmission is electrical in others only chemical or electrochemical one or the other being suppressed as required.

Denervated structures become more sensitive to neurohormones. This suggests that these neurohormones are continuously or very frequently secreted. In unstriated muscle increased sensitivity to an ion follows when the muscle is deprived of that particular ion such as calcium or potassium (Singh 1942, 1946).

#### SUMMARY AND CONCLUSIONS

1 The nature of response of frog's stomach muscle to nervous stimulation is described. The contraction is similar to that produced by acetylcholine and potassium and is not of the same type as that produced by alternating current suggesting that acetylcholine is liberated during nervous stimulation of frog's stomach. Excitation by nervous stimulation appears to involve the potassium ion.

2 Nervous stimulation also produces a contraction similar to that produced by alternating current thus suggesting that electrical transmission

precedes chemical. It is suggested that chemical transmission imparts tonic properties to the effects of electrical transmission.

3 On nervous stimulation, circular fibres of the stomach give the second kind of contraction, and longitudinal the first kind or tonic contraction. It is probable that the function of the longitudinal fibres is to maintain a tonic pressure on its contents and prevent the sagging of the stomach, and that of the circular fibres is to mix the contents by rhythmic contractions, as well as to exert a tonic pressure.

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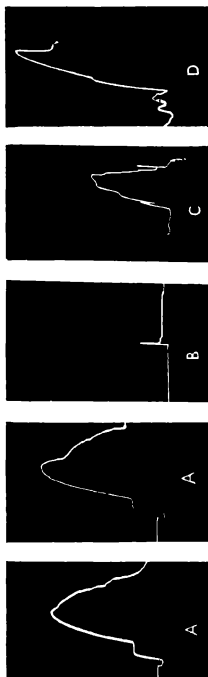


FIG. 2 Frog stomach. Action of eserine sulphate on the response to acetylcholine: (1 in 5000) A is normal response, B in 1 in  $10^2$ , C in 1 in  $10^3$ , D in 1 in  $10^4$

# INFLUENCE OF ROOT EXCRETIONS AND GERMINATING SEEDS ON NITROGEN-FIXATION BY AZOTOBACTER

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Received March 24 1947

In a previous paper (Uppal *et al* 1939) it was shown that *Azotobacter* from cultivated rice soils was stimulated to greater activity in fixing atmospheric nitrogen when grown in pure culture in association with growing roots of rice plants. This increased activity on the part of the micro-organism was further shown to be due to a change in reaction of the culture medium brought about by rice seedlings growing in it. In the experiments to be described below, the influence of the presence of growing roots of wheat, jowar (*Sorghum vulgare* L.) and radish on the fixation of nitrogen by *Azotobacter* in Ashby's cultures, has been investigated with a view to determining whether the living roots of these plants also stimulate the development of the micro-organism and thus enhance its nitrogen-fixing efficiency.

## EXPERIMENTAL METHODS AND RESULTS

For the purpose of inoculation, isolate III of *Azotobacter*, which has previously been shown to be an efficient nitrogen fixer (Uppal *et al*, 1939), was grown on Ashby's agar, and scrapings from a two days' growth of this micro-organism were used to inoculate the culture bottles containing 50 ml of Ashby's solution.

Seeds from which experimental seedlings originated, were disinfected in a one in 500 solution of corrosive sublimate for 15 minutes. These seeds were then washed in sterile water and aseptically removed to sterilised glass chambers lined inside with sterile, damp filter-paper. When the seedlings had made growths of 2 to 3 inches in length, 9 seedlings of each kind were transferred to culture bottles.

Four sets of culture bottles each containing 50 ml of Ashby's solution were prepared for each kind of plant and were treated in the following manner —

- (1) Inoculated with Is III,
- (2) Nine seedlings of each kind were grown in each culture bottle in this set,

(3) Same as in (2) above but each culture bottle was inoculated with Is. III, and

(4) Same as in (3) above, and in addition one gram of sterilised soil was added to each culture bottle.

At the conclusion of the experiment after 20 days, the total nitrogen was determined from duplicate samples of each culture bottle including the seedlings and soil. The results are given in Table I

TABLE I  
Influence of growing plant roots on nitrogen-fixation by *Azotobacter*  
(Total nitrogen in mg)

Ashby's solution containing	Radish		Rice		Wheat		Jowar	
	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average
9 seedlings	4.21	1.27	1.66	1.51	7.71	8.03	4.20	3.93
9 seedlings + Is. III	4.23	1.35	1.35	2.35	8.36	8.18	3.66	4.34
9 seedlings + Is. III + soil	6.89	6.37	2.27	2.44	8.71	8.20	4.30	7.31
9 seedlings + Is. III + soil	5.85	9.02	5.06	4.88	13.23	13.44	7.23	7.39
Net nitrogen fixed by Is. III in presence of seedlings and soil	8.61	3.17	1.70	3.83	1.80			

N.B. — Ashby's solution and soil in these experiments contained 0.06 mg N and 1.58 mg N, respectively. Is. III alone fixed 0.48 mg N.

Results in the above table show that, as reported earlier (Uppal *et al.*, 1939), *Azotobacter*, in association with living roots of seedlings alone or in combination with soil, was able to fix larger amounts of nitrogen than in the absence of such association. The organism was aroused to greatest activity when wheat seedlings were used, and fixed 3.83 mg. (13.44—(8.03+1.58)) nitrogen, followed closely by radish (3.17 mg. N). Rice and jowar were poor in this respect and exerted an almost equal degree of stimulation. It may be noted, however, that, when wheat seedlings were used alone without the soil, the organism did not fix, in such association, as much nitrogen as when radish was used. The significance of these results is not quite clear at present.

Hiltner, as quoted by Waksman (1931), found that "non-symbiotic nitrogen-fixation is stimulated by growing plant roots; the higher plants use up the available nitrogen in the soil and thus create a nitrogen-hunger

for the non-symbiotic nitrogen-fixing bacteria. The plants supply the bacteria with available energy, in the form of rotting root, hairs, root tips, etc." Waksman and Starkey (1931) came to a similar conclusion—"in the neighbourhood of growing roots of plants there is an excretion of soluble carbohydrates and addition of other residues to the soil which may serve as food for bacteria. Plants rapidly consume most of the available combined nitrogen from this portion of the soil. These two factors, namely, the presence of available sources of energy and a nitrogen minimum, would favour the rapid development of *Azotobacter* and *Clostridium* and lead to nitrogen fixation." Vyas (1934) working with maize seedlings, also noted that the maize roots excreted some stimulative product which enabled the non-symbiotic nitrogen-fixing micro organisms to fix larger amounts of nitrogen. Viswa Nath reported (1939) that the gain in the nitrogen-content of the soil under field conditions may be partly due to the stimulating action of root excretions on the nitrogen-fixing bacteria.

Whatever may be the contributing factors, it is obvious that the association of growing roots of plants with *Azotobacter* stimulates the development of the latter and leads to an enhanced activity on its part in fixing atmospheric nitrogen although it may be noted that the beneficial effect so exerted on the micro organism varies with the type of associated plant. It is claimed however, by some workers that sprouting seeds and living plants themselves have the power of fixing atmospheric nitrogen during germination and the subsequent growth of the seedling. Lipman and Taylor (1924) found that wheat and barley plants grown in culture solutions fixed nitrogen but it may be noted that no attempt was made in these experiments to maintain sterile conditions and that tap water was used for making culture solutions. Burk (1937), in controlled experiments with peas, did not obtain any evidence of nitrogen fixation during germination of pea seeds.

Sen (1929) has suggested the possibility that nitrogen fixing micro-organisms may live symbiotically in the roots of rice plants, whilst Viswa Nath (1932) holds the view that the rice plant itself has the power of fixing atmospheric nitrogen. The latter (1940) has also reported that maize seed, when germinated in a known volume of air devoid of all combined nitrogen, absorbed atmospheric nitrogen during germination of the seed and the subsequent growth of the seedlings. Jamieson as quoted by Winters (1924), claimed that all green plants possess the power of fixing nitrogen. On the other hand, Krassilnikov, as quoted by Lochhead (1940), has shown that *Azotobacter* was unable to grow in the rhizosphere of wheat, i.e., the subterranean part of the plant system, and was severely suppressed in that of maize. He attributed this to the toxic effect of root secretions.

In view of the conflicting evidence on the ability of growing plants to fix atmospheric nitrogen, an experiment was done to determine whether seeds absorbed nitrogen during germination and the subsequent growth of seedlings. Seeds of wheat, *jowar* (*Sorghum vulgare* L.) radish and pea, which were previously disinfected in a one in 500 solution of corrosive sublimate for 15 minutes, were germinated on damp filter-paper in moist, sterilised chambers at room temperature. Fifty seeds and an equal number of 4- and 20-day old seedlings of each kind of plant were analysed for total nitrogen. Results are presented in Table II and show that germinating seeds and young seedlings do not possess the power of fixing elemental nitrogen when tested under aseptic conditions.

TABLE II  
*Nitrogen-fixation by germinating seeds and seedlings*  
(Total Nitrogen in gm.)

Kind of plant	Seeds		4-day old seedlings		20-day old seedlings	
	50 seeds	Average	50 seedlings	Average	50 seedlings	Average
Radish ..	0.028 0.024	0.025	0.023 0.027	0.025	0.029 0.027	0.028
Rice ..	0.007 0.007	0.007	0.008 0.008	0.008	0.007 0.007	0.007
Wheat ..	0.049 0.063	0.051	0.048 0.048	0.044	0.044 0.036	0.040
<i>Jowar</i> ..	0.020 0.017	0.018	0.020 0.018	0.019	0.019 0.016	0.018
Pea* ..	0.197 0.174	0.185	0.184 0.185	0.184	0.187 0.177	0.182

\* In the case of peas, 25 seeds or seedlings were used

#### SUMMARY

In the presence of growing roots of wheat, radish, rice and *jowar* in Ashby's cultures, *Azotobacter* fixed larger amounts of atmospheric nitrogen than in their absence. The stimulating effect, however, varied with the kind of plant used, wheat exerting the greatest beneficial effect followed closely by radish. Rice and *jowar* were poor in this respect and exerted an almost equal degree of stimulation.

None of the seeds tested possessed any power of fixing elemental nitrogen during germination and the subsequent growth of the seedlings.

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## ADDITIONS TO FUNGI OF MADRAS—II\*

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Received March 22 1947

(Communicated by Rao Bihadur Dr B V Nath CIE DSC FRIC)

### (4) *Puccinia solani-giganteæ* sp. nov.

THIS rust is found on the leaves of *Solanum giganteum* Jacq. at Naduvattam, Nilgiris. Its presence can be recognized by the groups of orange yellow sori in the midst of the thick white tomentum on the lower surface of the leaves. Corresponding to these, small rusty brown spots are seen on the upper surface of the leaves.

Pycnia are developed towards the upper surface. They are subepidermal, globose, 100–180  $\mu$ , and yellowish brown in colour. Paraphyses and spores are present in the pycnia.

Aecia are hypophyllous, cup like, formed in groups one to three millimetres in diameter, each cluster having a variable number of sori. The aecium is sunk in the tissue of the leaf and is provided with a distinct peridium of one layer of thick walled warty cells (Fig. 1 b). The mean size of the aecium is 215  $\mu$  wide and 210  $\mu$  deep (range 200–270–180–225  $\mu$ ). Aeciospores are formed in chains from a basal hymenial layer of elongated cells as in other species of *Puccinia*. They are spherical or elliptic 17.8  $\mu$  (12–25  $\mu$ ) in diameter, very lightly coloured. The spore wall is hyaline with a finely warty surface.

Telia are hypophyllous and are formed mixed with the aecia as hard-rimmed, brown circular, open cups, 222  $\times$  202  $\mu$  (190–250  $\times$  180–215  $\mu$ ) in diameter and depth respectively. There is no distinct peridium as in the aecium, but the cup has a lining of two to three layers of small fungal cells. Teliospores are produced from the bottom of the cup (Fig. 1 c) on long hyaline pedicels which get easily separated from the spores. Teliospores are two-celled subspherical, rounded at the ends, slightly constricted in the middle, deep golden brown in colour and measure 37.9  $\times$  24.8  $\mu$ .

\* The first paper in this series appeared in *Proceedings of the Indian Academy of Sciences* Section B Vol XXV No 1. In this and succeeding papers it is proposed to describe the new fungi collected from various parts of the Presidency. New records of fungi will also find a place in these papers.

(28.52–20.28  $\mu$ ) The wall is smooth and uniformly thick. Some of the spores exhibit abnormalities having three or four cells by the formation of vertical or oblique cross walls in each cell (Fig. 1).

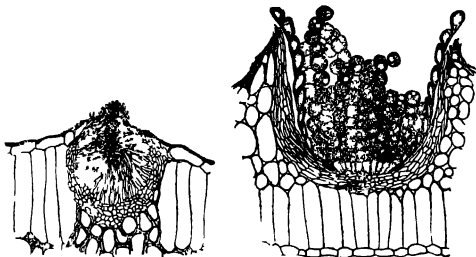
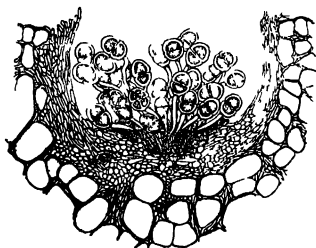


FIG. 1. (a) Pycnium of *P. solanacearum* ( $\times 360$ ). (b) Aetium of *P. solanacearum* ( $\times 360$ ).



(c) Telium of *P. solanacearum* ( $\times 360$ ).

**Identity of the rust.**—Several rusts have been described on *Solanum*. Most of these are from the Americas or West Indies. *Puccinia solanacearum* Sacc. et Syd. has been recorded from the Suttley valley in India. This produces O, I and III stages on *Solanum* sp. but unlike the rust under discussion the telia are formed only on the stem causing malformations.

*P. araucana* Diet et Neg forms I and III and even here the telia are formed only on the stem. *P. adducta* Arth has been recorded by Arthur (1918) on *S. racemosum* as having 0, II and III but not æcia. *P. tubulosa* Arth (*Aecidium tubulosum* Pat et Gaill) has been observed in India on *S. melongena* but the telial stage is found to develop on *Paspalum* sp. The other species of *Puccinia* on *Solanum* have been known to form only telia. *S. giganteum* has not been known to be infected by *Puccinia* till now. The peculiar cup-like telia are also characteristic and unlike those of other species on *Solanum*. Therefore it is concluded that this rust is different and is named *P. solani-giganteæ*. Dr G. R. Bisby to whom the specimens and the diagnosis were kindly forwarded by Dr B. B. Mundkur, is also of the opinion that this is an unrecorded species of *Puccinia*.

*Puccinia solani-giganteæ* sp. nov. — *Pycnia* epiphyllous, subepidermal, globoid, æcia in clusters, hypophyllous, cup-shaped  $210-215\mu$  with a distinct peridium of thick-walled warty cells, æcio-spores in chains, spherical or elliptical,  $17-8\mu$  ( $12-25\mu$ ), telia hypophyllous mixed with the æcia, cup-shaped  $220 \times 202\mu$ , teliospores 2-celled, deep golden brown, subspherical, smooth,  $37.9 \times 24.8\mu$  ( $28-52 \times 20-28$ ) stalked, stalks long hyaline, deciduous.

On living leaves of *Solanum giganteum* Jacq. Naduvattam (Nilgiris) 15-3-1946, Coll. C. L. Subramanian and K. Ramakrishnan (Type). Type specimen deposited in the herbarium of the Government Mycologist, Coimbatore, and Herb. Crypt. Ind. Orient, New Delhi.

*Puccinia solani-giganteæ* sp. nov. — *Pycnia* epiphylla, subepidermia, globosa, æcia aggregata, hypophylla, cupulata,  $210-215\mu$ , peridio distincto cellularum crasso-muratarum, æcio-sporidia catenulata globosa vel elliptica  $17-8\mu$  ( $12-25\mu$ ). Telia hypophylla æciis mixta, cupulata  $220 \times 202\mu$ , teliosporidia duo-cellata, aurati intense brunnei colores, subglobosa, lævia,  $37.9 \times 24.8\mu$  ( $28-52 \times 20-28\mu$ ), pedicellata, pedicelli longi, hyalini, decidui.

In vivis foliis *Solani giganteæ* Naduvattam (Nilgiris), 15 III 1946, Leg. C. L. Subramanian and K. Ramakrishnan. Typi specimina deposita in Herbario Government Mycologist Coimbatore et Herb. Crypt. Ind. Orient, New Delhi.

##### (5) *Entyloma bidentis* P. Henn

Saccardo-Syll. Fung. XIV, 495, 1888

On living leaves of *Bidentis pilosa* L. Coimbatore and Kallar (Coimbatore District) October 1946 (K. Ramakrishnan).

In October 1946, this smut was noticed in an epiphytotic form on the leaves of *Bidens pilosa*, a weed, in Coimbatore and Kallar. The lower leaves were first affected and later the infection spread to the upper also. Amphigenous, whitish to yellow, circular spots develop on the leaves. The spots are small in the initial stages but enlarge later becoming 0.5 to 1 cm. in diameter. They are isolated or sometimes coalescent. The upper surface of the spot becomes convex and the lower surface correspondingly concave. The colour deepens to yellow on the upper surface and finally turns brown

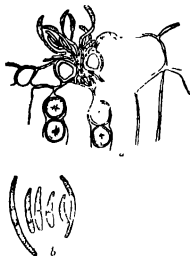


FIG. 2 (a) Section of leaf showing smut spores and conidia of *B. bidensis*. (b) conidia ( $\times 300$ )

The conidia of the smut are produced when the spots have attained their full size. These are formed on both sides of the spot, but first appear on the lower surface, producing a white-dotted appearance which may deepen into greenish yellow with age. The conidia are fusiform or filiform, hyaline, straight or bent, with one to three septa. The conidiophores emerge through the stomata in fasciculate masses and bear the conidia at their apices. The conidia measure  $7.8 \times 1.3 \mu$  ( $6-10 \times 1-2 \mu$ ). The conidial fructification is like *Entylomella* V. Hohn (Ciferri, 1928).

Inoculations were carried out on healthy plants of *Bidens pilosa* with these conidia. A suspension of the conidia was made in sterilised distilled water and this was brushed over the surface of the leaves after which the plants were kept covered with a bell-jar for 48 hours. On the fifth day small white spots developed on the inoculated leaves. These enlarged and in the course of fifteen days reached their maximum diameter. The colour of the spots deepened and brown streaks became evident in twenty days. At this stage conidial formation had commenced on the lower surface of

the spots and the smut spores also had developed within the leaf. Thus the relationship between the conidia and the smut was established.

The smut spores are spherical, ellipsoidal or angular and measure on an average  $15-13\mu$  ( $11-22-9-16\mu$ ). The epispore is smooth but sometimes a small hyaline appendage may be seen projecting from one angle. This is only the remnant of a hypha. The spores develop intercellularly in the palisade tissue forming chains, but in the spongy tissue of the mesophyll the spores are grouped together resulting in the displacement of the cells.

Conidia have been observed in a number of species of *Entyloma*, but they have not been described in the case of *E. bidentis*.

Saccardo has listed *E. bidentis* P. Henn. as occurring on *Bidens pilosa* in East Africa. The measurements of this smut as given by Saccardo are  $10-15 \times 9-14\mu$  with an epispore  $1-1\frac{1}{2}\mu$  thick. *E. guaranicum* Speg. has also been recorded on *Bidens pilosa* by Ciferri (1928). But the spores of this smut as described by Clinton (1904) are hyaline to light yellow with a prominent gelatinous envelope. The spores of the smut now recorded are brown and do not possess a gelatinous envelope. Owing to the difference in colour and the absence of the gelatinous envelope it is identified as *E. bidentis* P. Henn.

(6) *Entyloma dahliae* Syd.

Sydow, H. and P.

*Ann. Mycol. Berlin*, X, 36, 1912

Ciferri, R. de

*Ibid.*, XXVI, 56, 1928

Mundkur, B. B.

*Trans. Brit. Mycol. Soc.*, XXIV, 332, 1940

On leaves of *Dahlia variabilis* Desf. (garden variety), Ootacamund, Nilgiris, 30-9-1946 (T. S. Ramakrishnan).

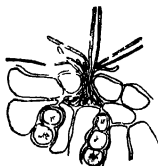


FIG. 3. Section of *Dahlia* leaf showing conidia of *E. dahliae* ( $\times 300$ ).

This smut is prevalent all over the upper elevations of the Nilgiris. Mundkur has stated that conidia were not found, nor has Sydow included

them in his original description. In the specimen collected from Ootacamund conidia were observed in large numbers. These were filiform hyaline, straight or bent. They were produced on conidiophores in fascicles emerging through the stomata on the upper surface (Fig. 3). The spots on which the conidia are formed exhibit a white ropy, reticulate appearance on the surface.

(7) *Melanotænium brachiaræ* Viegas

Syn *Tolyposporella brachiaræ* Mundkur and Thirumalachar, Mycological Papers, No. 16, Imp. Mys. Inst., London p. 5, 1946.

This smut has been observed in different parts of the Coimbatore District on the leaves and leaf sheaths of *Bracharia distachya* (L.) Stapf (*Panicum distachyum* L.).

Viegas has described *M. brachiaræ* on the leaves of *B. plantaginea* from Brazil. Mundkur and Thirumalachar have recorded *Tolyposporella brachiaræ* from Bangalore and New Delhi. Dr. Mundkur was kind enough to let us have fragments of the type specimens of these two fungi for comparison with the specimen collected at Coimbatore. There was close agreement between the three specimens. Microtome sections of the sori (Plate XVI, Fig. b) revealed that the spores occur in groups in the mesophyll tissue outside the ring of cells surrounding the vascular bundles. The spore germinates producing a promycelium from the apex of which a whorl of sporidia arise (Plate XVI, Fig. a).

A comparison of the type specimens of *M. brachiaræ* and *T. brachiaræ* with the local specimen showed that the external symptoms, the disposition of the sori and spores and the germination of the spores were alike in all the three. The measurements of the spores were as follows:

Specimen	Range of 1 mean n° of the spores in $\mu$	Mean size $\mu$
<i>M. brachiaræ</i>	8-14 $\times$ 6-14	11 $\times$ 8.1
<i>T. brachiaræ</i>	8-16 $\times$ 7-12	11 $\times$ 8.0
Local smut	8-17 $\times$ 6-11	11.5 $\times$ 9.0

It is clear from the above that the same fungus is involved in all these. It has to be decided whether it is an *Entyloma* or *Melanotænium*. The difference between these genera is very slight and rests mainly on the colour of the sori and the spores. A number of species described originally as *Entyloma* have been transferred to *Melanotænium* on this basis. Following this trend it is desirable to include this smut under *Melanotænium* on account

of the striae-like sori and dark olive-brown spores. Dr Mundkur's attention was drawn to the fact that the smut on *B. distachya* is not a *Tolypospora* but a *Melanotaniium* and he concurred with us in this.

(8) *Phyllachora cymbispora* sp. nov.

Spots forming yellowish green rings 1.2 mm wide surrounding the stroma, visible on both sides of the leaf, isolated or aggregated in groups of 2-4. Stromata solitary in each spot, clypeate black, shining, and raised almost equally on both sides of the leaf, 0.3-1.5 mm, with one locus, dense black just beneath the epidermal layers, locus broadly-flask-shaped and flattened, ostiolate, 225-396  $\mu$  broad and 162-228  $\mu$  deep, bounded by a cellular border, asci thin-walled cylindric-spindle-shaped, narrowed at the base, with a small foot, 120  $\times$  16  $\mu$  (102-150  $\times$  10-20.5), paraphyses present filiform, ascospores 8 light olivaceous, cymbiform, partly distichous, 30  $\times$  6.6  $\mu$  (22-38  $\times$  3.9  $\mu$ ), perithecia often present by the side of the perithecia subepidermal, deeply sunk dark-coloured, 123  $\mu$  broad and 180  $\mu$  deep, ostiolate, containing filiform, hyaline conidia (Plate XVI, Fig. c).

On living leaves of *Eurya japonica* Thunb., Lovedale (Nilgiris) 15-3 '46 K. Ramakrishnan and C. L. Subramanian, type. Type specimen deposited in the Herbarium of the Government Mycologist, Coimbatore and Herb. Crypt. Ind. Orient., New Delhi.

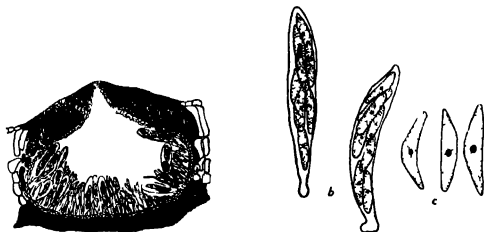


FIG. 4. (a) Section of perithecia of *Phyllachora cymbispora* ( $\times 100$ )  
(b) Ascus ( $\times 360$ ) (c) Ascospores ( $\times 510$ )

*Phyllachora cymbispora* sp. nov. Maculae formantes anulum flavidem viredem 1.2 mm lat., circumdantes stroma, utrumque visibiles, solitariae vel 2-4 aggregatae, stroma unum in quaque macula, clypeatum, nigrum,

micans, elevatum fere pariter utrimque folii 0.3-1.5 mm lat. uniloculatum, ostiolatum, stroma valde nigro infra epidermium, locusculus latiusculus, lageniformis, et compressus, 225-396  $\mu$  lat. 162-228  $\mu$  alt., cellis limbatus, asci tenuimuniti, cylindracei fusiformes unguistati in basi parvo pede, 120  $\times$  16  $\mu$  (102-150 10-20 5) paraphyses adsunt, filiformes, ascosporidia 8, lavis olivacei colores, cymbiformes partum disticha 30-66  $\mu$  (22-38  $\times$  3-9  $\mu$ ) pycnia saepe adsunt prope perithecia fuscicolores 123  $\mu$  lat. et 180  $\mu$  alt., ostiolata continentia filiformia conidia hyalina

In vivis foliis *Eurya japonica* Thunb., Lovedale (Nilgiris) Leg. K. Ramakrishnan et C. L. Subramanian, typus. Typi specimina deposita in Herbario Government Mycologist Coimbatore et Herb. Crypt. Ind. Orient., New Delhi.

*Catacauma eurya* (Racib.) Thuis et Syd. has been recorded on *Eurya acuminata* Dc. from Java and *Phyllachora transiens* Syd. et But. on the same host from Kumaon India. In both these fungi the stromata are hypophyllous. Further the asci and ascospores are smaller than those of *P. cymbispora*. Comparative measurements are given below.

	Asci	Ascospores
<i>C. eurya</i>	80-90 $\times$ 14-18 $\mu$	14-16 $\times$ 6-8 $\mu$ hyaline oval
<i>P. transiens</i>	80-70 $\times$ 10-11 $\mu$	20-22 $\times$ 6-7 $\mu$ hyaline oblong
<i>P. cymbispora</i>	102-150 $\times$ 10-20 $\mu$	22-38 $\times$ 3-9 $\mu$ light olivaceous cymbiform

It is manifest that the fungus under study is quite different from those mentioned above and is therefore named *Phyllachora cymbispora* (deriving the name from the shape of the spores).

(9) *Colletotrichum ciliatum* sp. nov.

Spots amphigenous, isolated, oval 4-9  $\times$  2-6 mm, or confluent forming irregular big patches, brownish grey with darker coloured margins, *acervuli* minute, numerous black, amphigenous separate, or confluent into linear striae, erumpent, stromata of dark brown cells filling the epidermis and one or two layers of subepidermal cells erumpent, setae numerous, 86-135  $\times$  5-10  $\mu$ , blackish brown, pointed 2-3 septate, *conidia* hyaline, unicellular, falcate, 29  $\times$  4.7  $\mu$  (19-25 3-5  $\mu$ ), with one terminal cilium, 5-19  $\mu$  long.

On living leaves of *Cymbopogon polyneuros* Stapf. Nanjanad (Nilgiris) 29-9-1946 (T. S. Ramakrishnan) Type.

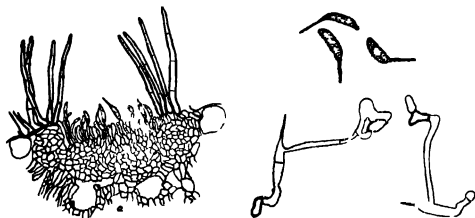


FIG. 5. (a) Section through the cervix of *C. ciliatum* ( $\times 240$ ) (b) Conidium ( $\times 510$ ) (c) Germinating conidium ( $\times 510$ )

*Colletotrichum ciliatum* sp. nov. — Maculae amphigeniae solitariae, ovals,  $4-9 \times 2-6$  mm, vel confluentes, formant, magna irregularia, panna, brunneolae, marginis fusciores, acervuli minutissimi, numerosi, fusci, amphigeni, solitariae, vel confluentibus cum lineari stris, erumpentis, stromata fuscarum brunnearum, cellarum, implentia, epidermidum et unum vel duo strata cellarum subepidermidum, erumpentia, setae numerosae,  $86-135 \times 5-10 \mu$ , fusci brunnei coloris, aculeatae, 2-3 septatae, conidia hyalina, unicellaria, falcata,  $29-47 \mu$  ( $19-25 \times 3-5 \mu$ ) cum uno cilio terminali.

In vivis foliis *Cymbopogon polyneus* Stapf Nanjanad (Nilgiris) 29-9 1946, leg. T. S. Ramakrishnan typus.

This species is unique in having a cilium at the apex of the conidium. It is in the form of a sharp process and is either straight or curved. The cilium is developed as the spore matures and is not evident in the earlier stages of spore development. The germination of the conidium is as in other species of *Colletotrichum*. One or more germ tubes are produced by the conidium and appressoria develop on these. A septum is seen in some of the germinating spores. The cilium is not shed during germination nor does a germ tube develop from it. The acervuli are first seen on the upper surface of the spots. They are formed on the lower surface only in the later stages. The infected leaves soon dry up owing to the formation of large lesions. On these dry leaves acervuli may be seen all over the surface.

The characteristics of the fungus show, that it is a *Colletotrichum*. But the conidia are ciliate—a feature not noticed in the genus. However the resemblance to *Colletotrichum* is so close that it is thought fit to include



- (a) Germinating spores of *Melantherium brachyrrhizon* ( $\times 600$ )  
 (b) Section through the sorus of *Melantherium brachyrrhizon* ( $\times 600$ )  
 (c) Section through the pycnidium of *Phyllosticta cyathosporea* ( $\times 400$ )



it in that genus, but as a new species *C. graminicolum* (Ces.) Wils. has been observed on *Cymbopogon* sp. but the conidia of this species are not ciliate. Therefore the present fungus is named *C. ciliatum*.

#### ACKNOWLEDGMENT

We wish to express our deep debt of gratitude to Dr. B. B. Mundkur of the Indian Agricultural Research Institute, New Delhi, for his ungrudging help in lending type specimens, helping with references to literature, and for critically going through the manuscript. We are grateful to Dr. Bisby of the Imperial Mycological Institute, Kew, for his help in the identification of the rust on *Solanum*, and to Rev. Fr. Singarayar of the St. Joseph's Seminary, Coimbatore, for rendering the diagnoses into Latin. Our grateful thanks are due to Mr. K. M. Thomas, Government Mycologist, for his constant help and encouragement. Mr. M. S. Balakrishnan, Research Fellow, was kind enough to prepare the drawings.

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## TWO SPECIES OF UNDESCRIBED LAMPYRID LARVÆ FROM S INDIA

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(Lecturer in Zoology Madras Christian College Tanburam)

Received April 16 1947

(Communicated by Prof S G M Ramanujam F A S C)

### I

*Luciola trivandriensis* sp n (Larva Trivandrum, 1942)

(Figs 1 and 2)

THE following notes are based on three hitherto unrecorded larvæ

*Length* 15 mm -20 mm

*Locality* —Trivandrum

*General colouration* —Dirty yellow

Body is strongly depressed and dorsal plates are flat with a median whitish longitudinal sulcus

Head is completely retractile into the thorax, about twice longer than broad, sides parallel, frontal sutures thickened into long ridges running parallel to each other up to the precoilar knobs, and metopic suture short and open. Antennæ are 3 jointed, with the basal joint very long, proximally membranous, middle joint slender, and apical joint minute with a sensory cone. Side by side with the apical joint is a sense papilla (Fig 2 s p), which is remarkable in being as long as the apical joint itself. The antenna of the present larva differs from that of *L. gorhami* (i) in having the membranous proximal part of the basal joint very extensive, (ii) in the basal joint lacking the basal sclerotisation, and (iii) in the apical sensory papilla being as long as the apical joint. Mandibles are falcate and canalliculate, the molar surface bearing the characteristic hairy fringe which is not so dense as in *L. gorhami*. The retinaculum which is subacute in *L. gorhami* is entirely wanting in the present species. The labiomaxillary plate does not show any striking difference.

Pronotum is longer than wide, anteriorly narrowed and slightly notched in front. Posterior margin is almost straight but with 2 pairs of posteriorly directed protuberances, the posterolateral pair being more rounded than the inner subdorsal pair. The inner pair of processes are continued anteriorly to a short distance as a pair of feeble carinæ. Mesonotum and

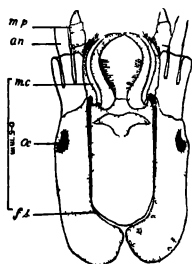


FIG 1

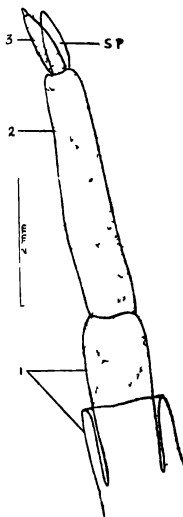


FIG 2

FIGS 1-2. FIG 1 *Lucila triandrensis* (larva)—head, an, antenna; fs, frontal suture; mandibular; ca, cal; mp, maxillary palpus; oc, ocelli. FIG 2 *L. triandrensis*—antenna 1, 2, 3 basal, middle and apical joints; fs, frontal suture.

metanotum are broader than long, with both pairs of processes and the subdorsal carinae. Abdominal terga 1-7 are all broader than long and have the two pairs of posterior processes. The subdorsal carinae form two parallel lines on the back of the larva. The eighth abdominal plate is subquadrate and without the middle pair of posterior processes but the posterior margin is widely emarginate. The ninth plate is very narrow and always bent under the eighth.

The three larvae collected could not be reared to the adult as they died soon. They were collected from a marshy locality in Trivandrum, S. Travancore in April 1942. I place these larvae provisionally under the genus *Luciola* because of their striking similarity to other described species of the genus in the occurrence of the posterior prominences but as it is impossible to get at the most probable species out of 31 recorded Indian species of *Luciola* I am driven to describe these larvae provisionally as species nova for purposes of description.

## II

(?) *Pyrophanes ndica* Mots

(Figs 3-5)

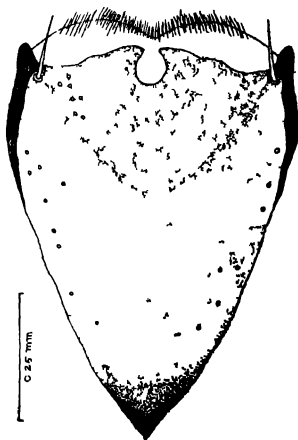


FIG. 3

Fig. 3 (?) *Pyrophanes ndica* Mots (larva)—Median plate of head capsule

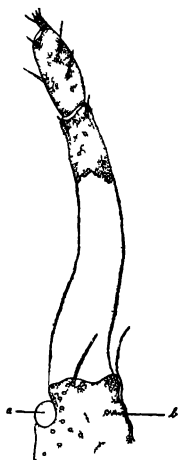


FIG 4

Fig 4 *P. dica* antenna (a) ocellus (b) head capsule (ventral view) (a) galea (b) palpus (c) stipes (d) alacardo (e) subcardo



FIG 5

Length—6 mm 17 mm

Locality—Tambaram

Colour—Black with castaneous patches

**Head** is chestnut brown with blackish shades. Epicranial suture is open throughout the frontal sutures running up to free margin of the head capsule which is thus divided into a median frontoclypeus and lateral parietals. Metopic suture is constricted at the middle. Frontoclypeus is roughly triangular with the base placed in front of the parietals. It is deeply sinuate anteromedially and thickened laterally forming an anterolateral precoila.

for mandibular articulation. Anteriorly the parietal gives off on the ventral side the mandibular apophyses which carry the acetabula for articulation with the condylar postartus of mandible. The hypostomal ridges possess a broad thickening near the middle. Ocelli are borne on open sockets. Antenna is 3-jointed, basal joint with a distal chitinous ring, the middle joint completely chitinised and terminal joint minute with 4 or 5 setæ. This larva resembles *L. gorhami* Rits. in its 3-jointed antenna, but differs from it in the absence of the basal sclerotisation and subglobular sensory papilla. In *L. tenebrosus* Wik. antenna is 2-jointed, the basal with very feeble chitinisation and apical with two sensory papillæ.

The buccopharyngeal apparatus includes the labrum, hypopharynx and pharynx. Labrum is semimembranous with its free margin dorsally visible and folded beneath to be attached dorsal to the base of hypopharynx. It consists of two pilose lobes separated by a notch and is strengthened by a minute central sclerite. Hypopharynx is a thin transverse membranous lobe lying in front of the pharynx close to the base of the mandibles. It is trilobed, the median lobe being suppressed and lateral lobes setose. The hypopharyngeal bracons are a pair of transverse chitinous bars united in the middle and attached to the hypostomal ridge close to the mandibular apophyses. Pharynx as usual presents two parts. Mandibles are falcate, canaliculate, basally dilated and setose and with a long seta just behind the external opening of the mandibular canal. It differs from *L. gorhami* Rits. and *Diaphanes* sp. in the absence of a sharp retinaculum.

At the base of the labiomaxillary plate is the transverse gular plate. Cardo is divided into a proximal narrow curved thick *subcardo* and a distal broad thin *alacardo* which is attached to the massive stipes. Stipes bears three long ventral setæ, two near the anterior border and one near the middle. Maxillary palpus tapers distally and is 4-jointed, basal stout with few long setæ, second transverse, third very narrow, and fourth conical. In *L. tenebrosus* Wik. the maxillary palpus is 3-jointed. The galea is 2-jointed, basal long and cylindrical with one or more long setæ and distal with one long apical seta besides a few short setæ. Lacinia is in the form of a dorsal densely setose cutting edge. Postlabial sclerite is long and deeply forked behind and carries two long symmetrically placed setæ. Each lobe of the bifid prelabium is provided ventrally with a pair of setæ, outer one being longer than the inner. Dorsally there is a pair of brush-like hairy growths which represent the paraglossæ. Labial palpus is 2-jointed.

**Thorax**—The ventral thoracic sclerites are ill-defined. On either side of the rudimentary prosternum is a slender obliquely placed cervical sclerite.

which gives off two long processes. Trochanter is undivided. Tergites are deeply arched without a mid dorsal sulcus and very slightly cleft anteriorly. Pronotum carries two castaneous fasciæ. Mesonotum and metanotum are black with a castaneous central area.

*Abdomen*.—Dorsally there are nine distinct tergites each with a pair of lateral lobes and anterolateral notches which are wanting or ill defined in the first and last tergites. All the tergites possess a median castaneous area. The lateral lobes also carry a similarly coloured area which in the first tergite is not clearly visible and in tergites 7, 8 and 9 it occupies almost the whole surface of the lobe. Ninth tergite carries posteriorly a single marginal row of from 10-16 stiff setæ. Abdominal tergites 2-8 have each a pair of conspicuous perforations,\* the nature and functions of which are obscure. Each pleurite is elongate and forked behind the dorsal or outer process of which being shorter than the setose ventral or inner process. The spiracle lies in the fork. In some larger specimens a second row of very minute pleurites are observed to lie between the sternites and spiracular pleurites. Sternites 2-6 are widely emarginate laterally and anteriorly, the anterolateral and posterolateral angles being produced. Sternites 7-9 are paler and less emarginate laterally. Ninth sternite carries four groups of setæ. Lying posterior to the ninth is an incomplete chitinous ring supporting the anal brush. Photogenic organs are paired and placed on the seventh abdominal segment. The pale green light is seen dorsally also through the pale lobes of the corresponding tergite. The anal brush consists of a circlet of creamy white retractile filaments, arranged in four bundles with four primary filaments in each bundle. Each filament is bifurcate and clothed with several minute recurved hooks.

*Internal Anatomy*.—The narrow œsophagus opens into the gizzard which leads into a long convoluted mid gut. The four Malpighian tubules form a coiled mass. A pair of connectives run from the brain to the sub-œsophageal ganglion which is more or less triangular. It is followed by three globular and equidistantly placed thoracic ganglia. Abdominal ganglia are eight. The metathoracic and first abdominal ganglia are not very much approximated. The last two ganglia are slightly approximated.

The special features of interest in the anatomy of the present larva are the open ocular fossa, divided cardo, complete division of the head-capsule, absence of mid-dorsal sulcus, deeply arched tergites, presence of marginal row of setæ in ninth abdominal tergite and the presence of lateral perforations in abdominal tergites 2-8.

---

\* These perforations are noticed in the living larva as minute creamy white specks.

This species is fairly abundant in certain months of the year especially in September and October. The larvæ were first collected by me in September 1941 from Tambaram. They were reared several times within the laboratory but most of them moulted once and then died. They were first mistaken for the very early stages of *L. tenebrosus* Wlk which are also available in the same locality. A detailed study of its morphology has revealed it to be entirely different from all the other lampyrid larvæ described so far. Most probably it is related to *Luciola* and belongs to the sub-family Luciolini. The only other Indian lampyrid genus of this sub-family is *Pyrophanes*, "the early stages of which appear to be quite unknown" (Blair, 1927). The only Indian species of this genus described is *P. indica* Mots.

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## INDEX TO VOL. XXV (B)

### AUTHORS' INDEX

- Daji, J. A.                    .. See Uppal and others.
- Ganju, P. N.                .. On *Beaniopsis rajmahalensis* gen. et sp. nov., a new type of gymnosperm female fructifications from the Jurassic of Behar, 95.  
                                   .. *Ontheanthus polyandra* gen. et sp. nov., a new type of fossil gymnosperm male fructifications from the Rajmahal Hills, 105.  
                                   .. *Ontheostrobilus sessilis* gen. et sp. nov., a new type of seed-bearing gymnosperm fructifications from the Jurassic of Onthea in the Rajmahal Hills, 119.
- Gnanamuthu, C. P.        . *Caligus sciænæ* N. sp. parasitic on *Sciæna glauca* from Madras, 43.
- Mahdihassan, S.         .. Specificity of bacterial symbiosis in aphrophorinæ, 155.
- Mahendra, Beni Charan .. Contributions to the bionomics, anatomy, reproduction and development of the Indian house-gecko, *Hemidactylus flaviviridis* Ruppel, Part IV, 57.
- Misra, P. L.                . On the three coccidian parasites *Wenyonella mackinoni* n. sp., *Eimeria lucknowensis* n. sp., and *Isoispora* sp., from the intestine of the wagtail *Motacilla alba* Linn. (*Passeriformes motacillidæ*), 75.
- Muthana, M. C.           .. See Singh and others.
- Nayar, R. Velappan      .. On the metamorphosis of two leptocephali from the Madras plankton, 1.
- Patel, M. K.                .. See Uppal and others.
- Raj, J. Samuel            .. Two species of undescribed lampyrid larvæ from S. India, 188.
- Ramakrishnan, K.        .. See Ramakrishnan and Ramakrishnan.
- Ramakrishnan, T. S.     .. Studies in the genus *colletotrichum*, III, 15.
- Ramakrishnan, T. S., and     Additions to fungi of Madras, I, II, 28, 178,  
     Ramakrishnan, K.

- Ramakrishnan, T S , and Soumini, C K      A new rust on *Premna tomentosa* Willd , 35
- Fruit rot of tomatoes caused by *Phytophthora palmivora* Butl , 39
- Rangappa, K S      Studies on the refractive index of milk, Part I, 86
- Singh, Inderjit, and Singh, (Mrs ) Sunita Inderjit      The mode of action of nerves on unstriated muscle, 163
- Singh, Inderjit, Singh (Mrs ) Sunita Inderjit, and Muthana, M C      (I) The interaction between ions drugs and electrical stimulation as indicated by the contraction of Avian unstriated muscle (II) Active elongation of unstriated muscle, 51
- Singh, (Mrs ) Sunita Inderjit      See Singh and others
- See Singh and Singh
- Soumini, C K      See Ramakrishnan and Soumini
- Uppal B N , Daji, J A , and Patel, M K      Influence of root excretions and germinating seeds on nitrogen fixation by azotobacter, 173

## TITLE INDEX

- Aphrophorinæ, specificity of bacterial symbiosis (Mahdihassan), 155
- Beaniopsis rajmahalensis* gen et sp nov , a new type of gymnosperm female fructifications from the Jurassic of Behar (Ganju), 95
- Caligus sciæna* N Sp parasitic on *Sciæna glauca* from Madras (Gnanamuthu), 43
- Colletotrichum, genus, studies, III (Ramakrishnan), 15
- Fungi of Madras, additions I, II (Ramakrishnan and Ramakrishnan), 28, 178
- Hemidactylus flaviviridis* Ruppel, Indian house gecko, contributions to the bionomics, anatomy, reproduction and development, IV (Mahendra), 57
- Ions drugs and electrical stimulation, the interaction between, as indicated by the contraction of Avian unstriated muscle (Singh and others), 51
- Larvæ, lampyrid, undescribed, from S India, two species (Samuel Raj), 188
- Leptocephali, two, from the Madras Plankton, on the metamorphosis (Nair), 1
- Milk, refractive index, studies. I (Rangappa), 86
- Motacilla alba* Linn (Passeriformes, Motacillidæ), wagtail, from the intestine, on three coccidian parasites *Wenyonella mackinnoni* n sp , *Fimeria lucknowensis* n sp , and *Isospora* sp (Misra), 75.
- Muscle, unstriated, the mode of action of nerves (Singh and Mrs, Singh), 163

Nitrogen-fixation of azotobacter, influence of root excretions and germinating seeds (Uppal and others), 173

*Ontheanthus polyandra* gen et sp nov , a new type of fossil gymnosperm male fructifications from the Rajmahal Hills (Ganju), 105

*Ontheostrobus sessilis* gen et sp nov , a new type of seed bearing gymnosperm fructifications from the Jurassic of Onthea in the Rajmahal Hills (Ganju), 119

*Phytophthora palmivora* Butl , fruit rot of tomatoes caused by (Ramakrishnan and Soumini), 39

Plant and animal breeding, statistical methods, symposium, 126

*Premna tomentosa* Willd , a new rust on (Ramakrishnan and Soumini), 35



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# CONTENTS

## SECTION B—VOL XXVI

### No. 1—July 1947

	PAGE
The Life-History of <i>Puccinia ruelliae</i> B & Br on <i>Ruellia prostrata</i> POIT MISS H SUNANDA KAMATH	1
Additions to Fungi of Madras III T S RAMAKRISHNAN AND K RAMAKRISHNAN	7

### No 2—August 1947

Studies in the Anatomy of Sugarcane Stalk I Chewing Canes K L KHANNA AND S L SHARMA	13
The Tapioca Plant and Methods for Evolving Improved Strains for Cultivation T K KUSHY	32
A New Rust on <i>Dalbergia paniculata</i> Roxb T S RAMAKRISHNAN AND K RAMAKRISHNAN	60
Revision of a Rust on <i>Oldenlandia</i> Spp T S RAMAKRISHNAN AND K RAMAKRISHNAN	64
Aeratron Affecting Growth and Sporulation of some Soil <i>Fusaria</i> in Liquid Cultures (MISS) T S SAROJINI AND (MISS) L YOGESWARI	69

### No 3—September 1947

<i>Fusarium</i> sp Parasitic on <i>Epipyrops</i> A Lepidopterous Parasite of the Sugar cane <i>Pyrilla</i> S Y PADMANABHAN	77
Studies on Sclerotium Forming Fungi—I <i>Sclerotium cepivorum</i> Berk and <i>S tuliparum</i> Klebahn Part 1 Cultural Studies R P ASTHANA	93
Studies on Sclerotium-Forming Fungi I <i>Sclerotium cepivorum</i> Berk and <i>S tuliparum</i> Klebahn Part 2 Symptoms Mode of Infection and Host Range R P ASTHANA	108
Studies on Sclerotium-Forming Fungi I <i>Sclerotium cepivorum</i> Berk and <i>Sclerotium tuliparum</i> Klebahn Part 3 Pectinase Activity and Preparation R P ASTHANA	117

### No 4—October 1947

Studies on the Refractive Index of Milk II Some Factors Affecting the Refractive Index and Refractive Constant of Milk K S RANGAPPA	125
The Natural Occurrence of Ergot in South India III T S RAMAKRISHNAN	136

	PAGE
<i>Phytophthora palmivora</i> Butler causing a Seedling Blight of <i>Hibiscus esculentus</i> L	M S BALAKRISHNAN 142
Studies in the Genus <i>Phytophthora</i> I Oospore Formation and Taxonomy of <i>Phytophthora palmivora</i> Butler	K M THOMAS, T S RAMAKRISHNAN C K SOUMINI AND M S BALAKRISHNAN 147
Embryogeny of <i>Isotoma longiflora</i> Presl	S B KAUSIK AND K SUBRAMANYAM 164
The Newly Hatched Larva of <i>Periclimenes (Ancylocaris) brevicarpalis</i> (Schenkel)	S GOPALAN NAYAR 168
No 5—November 1947	
Copepods of the West Hill Sea	P K JACOB AND M DEVIDAS MENON 177
Some Stages in the Development of the Pineal Complex of <i>Calotes versicolor</i> (Daud)	K K TIWARI 195
The Effect of the Interaction between Ions Drugs and Electrical Stimulation as Indicated by the Contraction of Human Unstriated Muscle	A K M KHAN AND INDERJIT SINGH 205
The Action of Direct Current on Unstriated Muscle	INDERJIT SINGH AND MRS SUNITA INDERJIT SINGH 211
No 6—December 1947	
Studies on the Embryology of Microphlebotomina Part I Reproduction and Breeding Seasons in the South Indian Vespertilionid Bat— <i>Scotophilus wroughtoni</i> (Thomas)	A GOPALAKRISHNA 219
Undescribed Males of Two Species of Gall Midges	K KARUNAKARAN NAYAR 233
Cytogenetical Studies in <i>Sesamum</i> Part I Cytology of the Parents, <i>Sesamum orientale</i> Linn and <i>Sesamum prostratum</i> Retz and the Cytology of the Sterile Hybrid between them and of the Fertile Amphidiploid	T S RAGHAVAN AND K V KRISHNAMURTHY 236

# THE LIFE-HISTORY OF *PUCCINIA RUELLIAE* (B & Br) ON *RUELLIA PROSTRATA* POIR.

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(Communicated by Dr T S Sadanvan, M sc, Ph D, F A sc)

SYDOW (1904) has recorded five species of *Puccinia* on the genus, *Ruellia*. He divided these into two groups one having verrucose-walled teliospores and the other smooth walled. Coming under the former are *P longiana* on *R tuberosa*, *P lateripes* (Berk and Rav) on *R ciliosa*, *P ruelliae-bourgaei* (D and H) on *R bourgaei* and *P ruelliae* on *R strepens* and *R prostrata*. *P paranahybæ* which has smooth-walled teliospores is recorded on *R longifolia*. The rust on *R prostrata* was from Ceylon while the others were from the Americas. Butler and Bisby (1931) have mentioned the occurrence of *P lateripes* on *Ruellia* spp at Pusa (Bihar) and *P ruelliae* on *R longifolia* (at Cawnpore) and *R prostrata* in various parts of India. Arthur (1934) has given six species of *Ruellia* serving as hosts for *P ruelliae* in the U S A. Further he has reduced *P lateripes* and *P ruelliae-bourgaei* to the status of synonyms of *P ruelliae*. This appears to be the correct view as there are no significant differences between the different species belonging to the group possessing verrucose teliospores.

Kellerman (1903) conducted successful infection experiments with *P lateripes* on *R strepens*. Arthur (1906) was able to produce infection on *R ciliosa* and *R strepens* with teliospores of *P lateripes*. *P ruelliae* is very common on *R prostrata* in and around Coimbatore in South India. It is a macro-cyclic and autoecious rust, all the stages being formed on the same host in the course of one and the same season. The succession of the different spore forms and their methods of development were closely studied. Advantage was taken of the occurrence of all the spore forms to follow the sequence in the development of the different stages by inoculation experiments. The present studies were carried out on *R prostrata* and the results are recorded in this communication.

*R prostrata* is a common perennial weed in South India which persists throughout the year in shady situations, but the rust is in evidence at

Coimbatore mainly from August to March of the succeeding year. During the south west monsoon in July and August the weed puts on fresh growth. The uredio and telial stages are seen in profusion at the beginning of this period. The lower leaves of the plant are the first to be infected. These sori are usually not found on the youngest leaves.

*Uredia*.—These are amphigenous and brown in colour. The sorus develops sub-epidermally. Later the epidermis is lifted up and finally ruptured. The hyphae of the fungus are intercellular, sending prominent twisted haustoria into the host cells. The uredial primordium forms below the epidermis and from this the urediospores are produced. These spores are stipitate, spherical to sub-globose, echinulate and yellowish brown in colour. Two equatorial germ pores are seen. Two nuclei are distinctly visible. The spores measure  $22 \times 23 \mu$ .



FIG 1 Young urediospores ( $\times 300$ )



FIG 2 Teliospores ( $\times 250$ )

*Telia*.—Soon after the formation of uredia, telia are formed often associated with uredia. Teliospores may develop either in urediosori mixed with urediospores or in separate sori. These are amphigenous, confined to the leaf-blades but more numerous than the urediosori and scattered all over the surface. They are dark chestnut brown, surrounded by the whitish flakes of the ruptured epidermal tissue. The development of the telia is sub-epidermal as in the uredia. Teliospores are pedicellate, the pedicel being hyaline and attached to the spore variously *i.e.*, to the base or obliquely to the side. The pedicel breaks easily leaving a portion of varying length persistently attached to the spore. Teliospores are two-celled chestnut brown, thick-walled, verrucose, sub-globose with rounded ends, slightly constricted at the septum and with one germ pore in each cell. The germ pore of the apical cell is at the top while in the lower cell it is placed variously. The spores measure  $24 \times 36 \mu$ .

Teliospores germinate readily without a period of rest. As a matter of fact, fresh spores collected in August, November and December exhibited a higher percentage of germination than those from specimens collected in

March Maneval (1922) found that the time required for the germination of teliospores varied with the period in which germination tests were made. It is less than two days in May, less than eight days in December, but over eighty days in September under conditions prevailing in Columbia. Under Coimbatore conditions, germination is evident in 12 to 24 hours when fresh spores are floated on drops of water on slides and kept inside a moist chamber. Germination was visible in hanging drops also in the spores which floated near the margin of the drops, while those which were immersed in water did not germinate. This indicates the necessity for aeration for teliospore germination. The promycelium emerges out through the germ pore. It is stout, hyaline and four-celled, the septa developing in the upper half of the promycelium. From each cell a sterigma is developed and on this an oval or round hyaline basidiospore is formed. Basidiospores commence germination even while they are attached to the basidium.

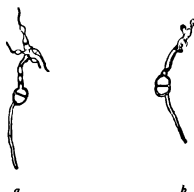


FIG 3 (a) Germinating teliospore showing the germination of basidiospores ( $\times 100$ )  
(b) Germinating teliospore ( $\times 100$ )

#### EXPERIMENTS AND OBSERVATIONS

Inoculations were made on healthy seedlings of *Ruellia prostrata* specially raised for the purpose and free from rust. Fresh teliospores were removed from sori and placed on drops of distilled water on the leaves or a suspension of fresh teliospores in sterilized water was brushed with a sterile camel-hair brush over the surface of the leaves, petiole and stem. The pots containing the seedlings were kept covered over with bell-jars to provide favourable conditions for infection and to prevent natural infection. Suitable control plants were also kept under similar conditions. Ten to twelve days after inoculation, groups of pycnia were evident on the inoculated leaves, petioles and stem. Younger leaves, petioles and stems were readily infected, while mature lower leaves or older portions of the stem

failed to take infection. This is explained by the fact that basidiospores are able to penetrate only younger tissues as the entry is effected by piercing the epidermal cell wall. On the leaf blades circular swollen areas develop which are convex either towards the upper or lower surface with a corresponding depression on the opposite side. The areas become studded with very minute red or yellowish brown pycnia on both sides. Pycnia are formed in abundance on the portions of the petioles and stem which are swollen as a result of hypertrophy. They are globose or oval sub-epidermal, paraphysate and measure on an average  $110 \times 190 \mu$ . The paraphyses and the spore stalks are elongated uninnuculate and light orange in colour. From the apex of each conidiophore oval to oblong pycniospores ( $4.5 \times 6.0 \mu$ ) are formed. These are hyaline or light pink in mass and float in the nectar which collects at the mouth of the pycnium. The paraphyses are prominent and project out of the ostiole. Aecia develop in the midst of groups of pycnia found on the leaf veins, petioles and swollen portions of the stem. The aecia are not formed on the interveinal portions of the leaf-blade though pycnial groups are present in these areas. The infected portions become swollen due to the hypertrophy of the tissues. In the leaf, the palisade cells of the mesophyll become enlarged. In the petiole and the stem the cortical cells are very much enlarged in the infected portions and contribute to the formation of the swellings. In severe cases of infection, clusters of branches like 'witches broom' develop from the nodes and numerous aecia are studded on the stem of these abnormal branches (Plate I, c).



FIG. 4. (a) Pycniospores ( $\times 150$ ) (b) Section of a portion of a young aecium ( $\times 300$ ) (c) Germinating aeciospore ( $\times 150$ )

**Aecia**—These are cupulate with a nearly cylindrical more or less white peridium with jagged edges, which soon become recurved. The aeciospores in mass are coloured deep brown. They are elliptic to irregular and thick-walled, the thickening being more pronounced at the apex and base,

prominently verrucose and measure  $22 \times 30 \mu$ . One conspicuous germ-pore is present.

The primordium of the æcium is first composed of a plectenchymatous mass of hyphæ formed three or more cell layers below the epidermis and which force the host cells apart. The host cells separate and in the resulting space the æcium develops. Its development is similar to what is observed in many of the cupulate æcia. The binucleate nature of the cells is evident in the basal cells of the hymenium and also in many of the hyphal cells found much below the hymenium (see Fig 4 b). The peridium is made up of one layer of cells which forms a continuous envelop. extending from the sides and arching over the young æcium. Later the host tissue is pushed up and eventually ruptured exposing the æcium and the æciospores. The æciospores are developed from the basal cells in chains. The æciospores alternate with intercalary cells which shrivel and disintegrate, thus facilitating the dismemberment of the æciospores from the chains. Aeciospores are capable of immediate germination. One germ tube is produced from each spore which grows out through the germ pore. Viability of the æciospores is gradually lost with age. Fresh æciospores germinate in twelve hours but æciospores from specimens which had been kept between drying sheets and stored in envelopes at laboratory temperature ( $26-28^{\circ} \text{C}$ ) for three months did not exhibit any signs of germination even after two days under optimum conditions. In December, suspensions of fresh æciospores were brushed on the surface of leaves of healthy *Ruellia* seedlings and these were covered over with bell-jars to provide humid conditions. In fifteen to twenty-two days uredia developed on the inoculated leaves. Unlike the pycnia produced from basidiospores, the urediosori were formed only on the older lower leaves and failed to infect younger leaves at the apices of the shoot. The entry of germ tube from æciospores being through the stomata, the older leaves are readily infected. In the course of another week, teliosori were also observed on the same leaf. The control plants were free from infection.

The above studies have shown that *P. ruelliae* is a macro-cyclic, autoæcious eu-form of rust exhibiting 0, I, II, and III stages. Starting with the teliospore the life-history has been followed up through all the other stages. During these studies it was found that during one season this rust can complete two or more cycles on this host.

#### SUMMARY

The life-history of *Puccinia ruelliae* on *Ruellia prostrata* was studied by inoculation experiments. It is a macro-cyclic and autoæcious rust, all the

stages being formed on the same host in one and the same season. The succession of the different spore forms and their method of development were closely studied. Advantage was taken of the occurrence of all the spore forms to follow the sequence in the development of the different stages by inoculation experiments.

#### ACKNOWLEDGMENTS

My thanks are due to Mr T S Ramakrishnan, Assistant Mycologist, Agricultural College and Research Institute, Coimbatore, for helpful criticisms and suggestions. I am also grateful to Mr K M Thomas, Government Mycologist, Agricultural College and Research Institute, Coimbatore, for having gone through the manuscript and rendering necessary help to carry out this piece of investigation.

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- A Section through a pyrenium showing paraphyses and the pyreniospores  
 B Section through an ascium showing the hymenial layer, peridium and the ascospores  
 C Showing cluster of branches like witches broom and the numerous cysts studded on the branches



## ADDITIONS TO FUNGI OF MADRAS—III\*

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Received May 26 1947  
(Communicated by Dr T S Sadasivan M Sc Ph D, F A Sc)

### (10) *Physalospora pterolobii* Ramakrishnan, T S and K sp nov

Spots circular isolated epiphyllous, yellowish green with black centre, *perithecia* one to four in a spot, immersed in the tissue, deep-seated, globose ostiolate,  $290 \times 266 \mu$  ( $259-335 \times 222-315 \mu$ ), peridium of two to three layers of dark brown cells, thicker at the apex near the ostiole, *asci* cylindric-elongate,  $107 \times 14 \mu$  ( $85-136 \times 9-19 \mu$ ), produced from the base and the sides, 8-spored, *ascospores* oblong, one-celled, hyaline, uniseriate,  $13 \times 5 \mu$  ( $11-18 \times 4-7 \mu$ ), paraphysate, *paraphyses* filiform, *pycnidia* immersed in the tissue, associated with the perithecia, globose, containing minute, hyaline, rod-shaped, spores. The pycnidium resembles a spermatogonium.

On living leaves of *Pterolobium indicum* A Rich, Kallar (Coimbatore district) 9-X-1946, T S Ramakrishnan

Maculae orbiculares, epiphyllae, *perithecia* 1-4 per maculam, subepidermia, globosa, ostiolata, paraphysata, *paraphyses* filiformes, *asci* elongati-cylindrici,  $107-14 \mu$ , octosporiati, *ascosporidia* oblongata unicellata, hyalina, uniseriata,  $13 \times 5 \mu$ , *pycnidia* subepidermia, globosa, prope *perithecia*, *pycnidiosporidia* minuta, hyalina, baculoformia.

In vivis foliis *Pterolobi indicum* A Rich, Kallar (Coimbatore) 9-X-1946, T. S. Ramakrishnan

The leaf is thickened at the region of infection due to the enlargement of the mesophyll cells

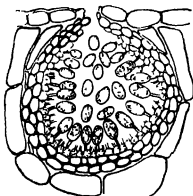
\* Part I of this series was published in *The Proceedings of the Indian Academy of Sciences*, January 1947, 25 No 1

Part II in the same Journal, June 1947, 25, No 6

The type specimens have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and Herb Crypt Ind Orient, New Delhi

(11) *Physalospora heterostemma* Ramakrishnan, T. S. and K., sp. nov.

*Perithecia* epiphyllous, gregarious, groups scattered, 4-10 or more in each group, innate, extending up to the spongy parenchyma, erumpent, dark-brown to sepia, ostiolate, peridium of two to three layers of thick-walled dark-brown, polygonal cells,  $178 \times 170 \mu$  ( $155-203 \times 150-185 \mu$ ), paraphysate, paraphyses linear and septate, asci clavate, hyaline, 8-spored  $74 \times 13 \mu$  ( $64-93 \times 7-15 \mu$ ); ascospores irregularly uniseriate, one-celled oblong, hyaline,  $15 \times 7 \mu$  ( $11-19 \times 6-9 \mu$ ); pycnidia of two kinds, occurring near the perithecia; one type of pycnidium immersed, globose,  $111 \times 111 \mu$  ostiolate, with a dark brown, two to three layered peridium; pycnidiospores oval, hyaline, one-celled, and resemble those of *Macrophoma*; second type of pycnidium also globose, immersed, in the tissue, producing large numbers of minute, hyaline, rod-shaped pycnidiospores. This structure resembles a spermatogonium (Plate II, c).

TEXT-FIG. 1. Pycnidium (*Macrophoma* stage)  $\times 400$ 

On living leaves of *Heterostemma tanjorensis* W. and A., Kallar (Coimbatore district), 9-X-1946, T. S. Ramakrishnan and K. Ramakrishnan.

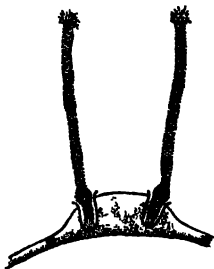
*Perithecia* epiphylla, gregaria, subepidermia, erumpentia, ostiolata,  $178 \times 170 \mu$ ; asci clavati, hyalini,  $74 \times 13 \mu$ , octosporiati; ascosporida, irregulariter uniseriatea, oblongata, hyalina,  $15 \times 7 \mu$ ; paraphyses adsunt murus asci gelatinous in aquæ; 2 genera pycnidium, 1) immersa, globosa, pycniosporidia hyalina, ovalia unicellata, 2) globosa, pycniosporidia minuta, hyalina, baculo-formia.

In vivis foliis *Heterostemma tanjorensis* W. and A., Kallar (Coimbatore district) 9-X-1946, T. S. Ramakrishnan et K. Ramakrishnan.

The asci start from the base of the perithecium and when mounted in water exhibit intense gelatinisation of the wall and consequently a clear translucent area is seen between the spore and the outer wall of the ascus.

(12) *Kernia lauricola* Thirumalachar

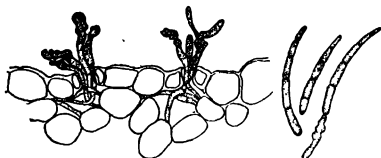
This was collected on two hosts belonging to the Lauraceæ—*Phoebe paniculata* Nees and *P. Wightii* Meisn., from several places in the Nilgiris (Ootacamund Coonoor and Naduvattam). Only the telial stage was available. The telia are columnar and are produced in a circle from the margins of small circular swellings on the lower surface of the leaves (Plate a). Six to twelve such columns are produced in each ring. The telium takes its origin from a layer of long parallelly arranged hymenial cells at the bottom of a cup-like depression. The teliospores are stipitate, two-celled with long stalks. The columnar structure is produced by the plating together of the stalks of the raised teliospores. The teliospores measure  $35 \times 22 \mu$  ( $26-42 \times 16-28 \mu$ ). The two cells of the teliospores are almost equal in length. They are deep chestnut brown in colour and have smooth walls. There is a constriction between the two cells. This rust closely resembles the species recorded by Thirumalachar (1946) on *Litsea* sp.

TEXT FIG. 2. Telia of *K. lauricola*  $\times 100$ (13) *Cercospora adinae* Ramakrishnan, T. S. and K., sp. nov.

Spots hypophyllous, without any definite outline, forming irregular often confluent ochraceous-orange patches, involving much of the leaf surface, mycelium internal septate, *conidiophores* hypophyllous, densely tufted, emerging through the stomata branched or unbranched, filled with deep orange contents and repeatedly geniculate at the apices, *conidia* elongate, broad below the middle, and tapering towards the apex, base

flattened, apex rounded, straight or curved, 3-7 septate,  $54-84 \times 4-7 \mu$ , contents hyaline to orange.

On living leaves of *Adina cordifolia* Hook., Walayar (Malabar district) 31-XII-1946, T. S. Ramakrishnan and K. Ramakrishnan.



TEXT-FIG. 3. Conidiophores and conidia of *Cercospora adinae*  $\times 400$ .

Panni hypophylli, silacei-lutei colores, conidiophora dense fasciculata, emergentia per stomata, septata, contents dense lutea, conidia elongata, obclavata, recta vel curva, 3-7 septata, hyalina vel lutea,  $54-84 \times 4-7 \mu$ .

In vivis foliis *Adina cordifolia* Hook. Walayar (Malabar district) 31-XII-1946, T. S. Ramakrishnan et K. Ramakrishnan.

This fungus does not form definite spots on the leaves and often the incidence of the infection cannot be detected from the upper surface. Since this fungus is found to be different from the others recorded on plants belonging to the family Rubiaceae it is described as a new species.

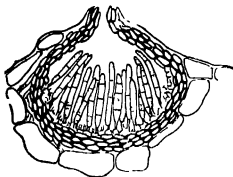
(14) *Septoria erythrinae* Ramakrishnan T. S. and K., sp. nov

Spots numerous, small, angular, bounded by veinlets, light green in colour; pycnidia hypophyllous, 2-5 in a spot, black, subepidermal, immersed in the tissue, slightly erumpent, globose, ostiolate,  $150 \times 90 \mu$ , with a peridium of two to three layers of brown cells; pycnidiospores long, cylindrical,  $44 \times 4 \mu$  ( $36-58 \times 4-6 \mu$ ), straight, three-septate, hyaline produced on very short stalks.

On living leaves of *Erythrina* sp. Kallar (Coimbatore district) 9-X-1946 T. S. Ramakrishnan and K. Ramakrishnan.

Maculae parvae, angulares, leviter virides; pycnidia hypophylla, 2-5 per maculam, nigra, subepidermia, globosa, ostiolata,  $150 \times 90 \mu$ ; pycnidiosporidia cylindrica, recta, triseptata, hyalina, brevipedicellata,  $44 \times 4 \mu$  ( $36-58 \times 4-6 \mu$ ).

In vivis foliis *Erythrina* sp. Kallar (Coimbatore district) 9-X-1946; T. S. Ramakrishnan et K. Ramakrishnan.



TEXT-FIG 4 Pycnidium of *Septoria erythrinae*  $\times 400$

The spores come out of the ostiole in the form of thread-like whitish masses. When examined with a lens these can be readily made out in fresh specimens as white peg-like projections from the pycnidia ('spore horns').

*Phlyctana brunneola* (Berk.) Sacc. (*Septoria brunneola* Berk.) has been described on dead branches and stem of *Erythrina crista-galli*. Saccardo does not give the measurements of the pycnidiospores or the pycnia. The fungus under study is however found parasitic on living leaves of *Erythrina* sp. and is not a *Phlyctana*. It is undoubtedly new to science and is proposed as a new species.

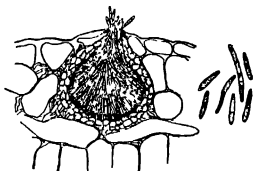
(15) *Septoria thespesiæ* Ramakrishnan T. S. and K., sp. nov

Spots circular, amphigenous, isolated or confluent, 4-15 mm. in diameter, upper surface blackish brown with grey centre and lower surface sepia coloured; *pycnidia* globose, innate, immersed in the tissue, numerous in the spot,  $88 \times 93 \mu$  ( $74-96 \times 63-111 \mu$ ), peridium of two to three layers of small cells, *pycniospores* straight cylindrical with tapering ends 2-6 septate, hyaline, borne on very short stalks,  $28 \times 4 \mu$  ( $9-37 \times 2-4 \mu$ ).

On living leaves of *Thespesia populnea* Cav. Coimbatore, 18-ii-1947, T. S. Ramakrishnan and K. Ramakrishnan.

Maculae orbiculares, amphigenae; *pycnidia* globosa, subepidermia, ostiolata; *pycniosporidia* recta, cylindrica, cum terminis angustitatis, 2-6 septata, hyalina, brevipedicellata,  $28 \times 4 \mu$  ( $9-37 \times 2-4 \mu$ ).

In vivis foliis *Thespesia populnea* Cav. Coimbatore, 18-ii-1947, T. S. Ramakrishnan et K. Ramakrishnan.



TEXT-FIG. 5 Pycnidium of *Septoria thespetiae*, pycnidiospores  $\times 300$

This is very common on *Thespesia populnea* throughout the province all through the year. The spots become almost black on old yellow leaves. The pycnidiospores come out in masses as 'spore horns' through the ostiole of the pycnidium.

#### ACKNOWLEDGMENT

The authors are grateful to Dr. B. B. Mundkur of New Delhi for going through the manuscript critically and to Mr. K. M. Thomas, Government Mycologist, Coimbatore, for his constant interest and encouragement. We are also thankful to Mr. M. S. Balakrishnan, Research Fellow, for making some of the drawings, and to Rev. Fr M. Singarayar of St. Joseph's Seminary, Coimbatore, for the Latin translations of the diagnoses.

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*l*



- a* Leaf of *Phoebe paniculata* showing the telial columns of *Kernia lauricola*  
*b* Section of a perithecium of *Physalospora heterostemma*  
*c* Section of a spermatogonium of *Physalospora heterostemma*



# STUDIES IN THE ANATOMY OF SUGARCANE STALK

## I Chewing Canes

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Received October 1 1946

## I INTRODUCTION

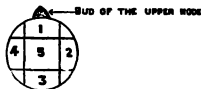
THE study of chewing canes was taken up because of their economic importance in urban areas. In the vicinity of large towns, there is always a great demand for them, and a small farmer with irrigation facilities and adequate manuring can get a remunerative return for his investment if he puts in the market, a really suitable variety for sale. Apart from the sweetness, the thickness and the colour of the cane, the ease with which it can be peeled and crushed by the human jaw, is the primary consideration desired in a chewing cane. The latter features naturally call for a detailed study of the anatomical make up of the stalk as a whole to enable differential ratings in respect of the rind and core tissues in different varieties to be adequately assessed. While the internal structure of sugarcane stalk was studied in some detail by Bremekamp (1914) and Artschwager (1925), no serious attempt seems to have been made till recently to work out the comparative anatomy of different varieties and correlate it with features of economic importance. Ueno (1938) demonstrated that rind hardness was closely associated with the number or the size of vascular bundles. Khanna and Panje (1939) found that rind hardness was not a simple function of vascular bundles alone. According to them, the lignification of parenchymatous matrix and vascular sheaths was also a major factor involved in determining the degree of rind hardness. Buzacott (1940) and Rao (1941) showed that anatomical structure of a cane variety as reflected in the rind hardness contributed much towards its resistance to stem-borers.

The present contribution is an attempt to understand what anatomical features are a pre-requisite in a cane stalk to be suitable for chewing purposes.

## II MATERIAL AND METHODS

Three well-known varieties of chewing canes, namely Saharanpur Paunda, Amritsar Paunda and Peshawar Paunda, were selected for this study. The upper half of middle internodes of three representative stalks

formed a sample for each variety. Since it was not possible to get intact, an entire cross-section of so thick a cane, the internode was longitudinally split into four peripheral and one central sectors which were numbered as shown below



Hand sections from each sector were stained with 1% solution of Safranin in 50% alcohol, and after the usual process of dehydration, were mounted in Canada Balsam.

The following characters were studied —

#### A. Vascular bundles—

(a) *Number per unit area*—All the vascular bundles occurring in 3 unit-areas were counted in each sector, the field of the microscope being taken as a unit. In order that the counting was done in regions comparable to one another, the edge of the field of microscope, was kept touching the epidermis in sectors 1 to 4 and for the 5th sector, innermost portions were taken. There were 36 countings for rind and 9 for central region for each variety.

(b) *Size*—Because of the wide variations in the size of the vascular bundles occurring in the rind region and the inherent difficulty in selecting them without any bias, it was measured for all of them found in the field of the microscope, for each unit area, in terms of their two axes, radial and tangential, with respect to the cross-section of the cane. In the central region where the variability in their size was rather low, the size of only six per field which amounted to nearly 50–65% of the population, was measured. Thus 358 vascular bundles were measured in the peripheral sectors of the Saharanpur Paunda, 369 for the Peshawar variety and 465 for the Amritsar Paunda. For the central region, 54 observations were made for each variety.

In view of the basic pattern of a quadrilateral figure symmetrical along its radial axis, to which all the shapes of vascular bundles conformed, it appeared that the product of the two axes would give a very reliable index of the size of vascular bundles in different varieties for a comparative study. To test this hypothesis, size of all the vascular bundles in a unit area (1.9 mm in diameter) in each of the four peripheral sectors of all the three canes in each variety was determined by the following three methods —

(i) The radial and tangential axes of vascular bundles were measured under microscope in terms of the divisions of eye-piece micrometer, product of the two giving a comparative value of the size

(ii) Those very vascular bundles were drawn 140 times magnified with the help of a camera lucida. Product of the two longest axes of the figure at right angles to each other and measured to the nearest mm was taken as an index of the area of the figure

(iii) The actual area of the figure was measured with a planimeter to the nearest 0.25 sq. cm. As this obviously bore the most accurate relationship to the actual respective areas of the vascular bundles, it was taken as the standard with which the two sets of indices mentioned above had to be compared

Statistical analysis of these three sets of data for 517 vascular bundles (193, 164 and 160 vascular bundles respectively of Amritsar, Saharanpur and Peshawar Paundas) showed that there existed a very high positive correlation of the order of 0.9546 between (i) and (iii) and 0.9699 between (ii) and (iii)

Similar determinations for 90 vascular bundles taken at random from central sectors, 10 from each gave +0.8889 as coefficient of correlation between (ii) and (iii) methods. The coefficient of correlation between (i) and (iii) was not determined because of the close correspondence found to exist between this value and that for (ii) and (iii) in respect of the vascular bundles of the peripheral region

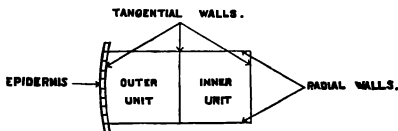
The method (i) followed for determining the size of vascular bundles was, therefore, quite reliable for a comparative study. Besides the method was both quicker and less laborious, thus enabling a large number of measurements to be taken. A further contribution on the subject will, however, be made separately.

(c) *Total area* occupied by vascular bundles in a unit area in peripheral region was found by adding the size of all of them and in the case of the central sector by multiplying the average size (calculated from that of six bundles in a particular unit) by their number in that unit.

#### **B. Parenchymatous cells—**

In a unit area (square in shape), the number of these cells, which obviously, was inversely proportional to their average size and was therefore an index of the latter, was calculated by multiplying the number of cells situated on the two adjoining sides of the square in the case of the 5th sector where total area under vascular bundles in a unit area, was not

significantly different in these varieties. To get the requisite figure for peripheral sectors, where cells rapidly increased in size as one proceeded from the epidermis towards the centre, the average of the number of cells situated on the opposite sides of the square (tangential in respect to the cross-section of the cane) was multiplied with that on the radial. For the outer unit, epidermis formed the outer of the two (tangential as stated above) sides of the square while the inner unit was bounded towards the periphery by the inner side of the outer unit as shown in the diagram below. The area occupied by the vascular bundles did not interfere with such determinations because parenchymatous cells in this region were arranged more or less in layers. Actual counting of cells in a unit area or the determination of cell-size by planimeter did not seem to be a practical proposition.



36 observations each for the outer and inner units, were made in the peripheral region and 9 for the core for each variety.

### C Thickness of cell-walls—

This was measured for two adjoining cells in the parenchyma at 0.6 mm and 1.2 mm from the epidermis and in the centre. Similarly for the sclerenchyma, it was noted in the sheaths of 2–3 vascular bundles, the biggest of them in the field. In rind, 120 readings were taken at each depth for parenchyma and sclerenchyma and 30 only for each tissue in the core of a variety.

## III OBSERVATIONS AND DATA

From the foregoing paras, it would appear that the cross-section of a cane stalk was studied more or less completely for all the anatomical features which were responsible for the hardness of a cane. They are listed below.

(A) The number, the size and the total area of vascular bundles in a unit area and the thickness of walls of cells forming their sheaths.

(B) The number of parenchymatous cells in unit area and the thickness of their walls, the former being an index of their size.

Since the total area occupied by the vascular bundles was a function of their number and size, and included these two characters in their entirety, a separate consideration was not given to them as such, thus leaving for critical examination, six characters for rind, viz (i) the area under vascular bundles, (ii) number of parenchymatous cells in outer unit area and (iii) in inner unit area, (iv) the thickness of cell-walls in parenchyma at 0.6 mm and (v) at 1.2 mm and (vi) the thickness of cell-walls in the vascular sheaths, and four characters for the storage tissue, namely (i) total area of vascular bundles, (ii) number of parenchymatous cells in unit area and (iii) thickness of cell walls in parenchyma and (iv) in sclerenchyma

*Rind*—In the formation of rind, the Amritsar Paunda showed the greatest development of five characters, viz, the total area of vascular bundles, the thickness of cell-walls of sclerenchyma, and parenchyma at 0.6 mm depth and the number of parenchymatous cells in both the outer and inner units (Table I) in all of which except the last named character it was significantly different from the other two varieties at 1% level (Plate III, Figs 1-3). The difference between this variety and the Peshawar Paunda in respect of this character was significant at 5% level only. The size of the parenchymatous cells being inversely proportional to their number in unit area, the difference in their size was clearly visible where their number in two varieties was significantly different, the higher the level of significance, the more pronounced the difference in their size. In Amritsar Paunda cells forming the parenchymatous matrix were smallest of all the three varieties (Plate IV, Figs 1, 2, 3 and Figs 4, 5, 6). The thickness of parenchymatous cell-walls at 1.2 mm from the epidermis was just a little less than that in the Peshawar Paunda, the difference being insignificant even at 5% level. It is interesting to note that in these two characters also it was significantly different from the Saharanpur variety at even 1% level. The latter exhibited the lowest magnitude (Plate III, Fig. 1) for characters 4, 5, 6 and 7 and was significantly different at 5% level from the variety nearer to it in the three underlined characters and at 1% level in two only namely the 6th and the 7th character. As regards characters 3 and 8, it occupied an intermediate position and was significantly different in respect of the 3rd character only. The ground tissue in this variety was, therefore, composed of largest cells with thinnest walls (Plate IV, Figs 1, 2, 3 and Figs 4, 5, 6).

The Peshawar Paunda was situated in between these two varieties so far as characters 4, 5 and 6 were concerned, and was significantly different in the 4th and the 6th characters at 5% and 1% level respectively. In respect of the 3rd and the 8th characters it was least developed but showed maximum development for the 7th character. It was significantly different in the 3rd

TABLE I

Showing various anatomical features of 3 varieties of chewing canes

Characters	Varieties	Saharan pur Paunda	Peshawar Paunda	Amritsar Paunda	General mean	Critical difference at	
						1%	5%
<i>Rind Peripheral Region</i>							
<i>A Vascular bundles</i>							
(1) Number per unit area	..	9.94	10.25	12.92	11.04	1.33	1.00
(2) Size		177.7	147.4	170.9	166.7		
(3) Total area occupied by them in a unit area		1767.4	1511.0	2207.8	1828.8	148.5	112.5
<i>B Numbers of Parenchymatous cells</i>							
(4) In outer unit area		885.2	902.3	1156.3	974.6	43.5	33.1
(5) In inner unit area		138.0	140.0	147.1	142.1	8.35	6.35
<i>C Thickness of cell walls</i>							
<i>(i) Parenchyma</i>							
(6) At 0.6 mm		8.00	8.81	9.72	8.84	0.57	0.43
(7) At 1.2 mm		6.81	8.16	8.10	7.69	0.46	0.35
<i>(ii) Sclerenchyma</i>							
(8) In sheath		18.19	17.56	21.96	19.24	1.63	1.24
<i>Storage tissue Central Region</i>							
<i>A Vascular bundles</i>							
(9) Number per unit area		11.22	11.60	9.22	10.07	1.77	1.30
(10) Size		274.6	334.7	401.9	337.1	47.3	36.00
(11) Total area occupied by them in a unit area		3089.4	3684.7	3628.2	3455.4	1125.4	630.5
<i>B Parenchymatous cells</i>							
(12) Number per unit area		121.8	160.0	142.0	141.3	14.69	10.68
<i>C Thickness of cell walls</i>							
<i>(i) In parenchyma</i>							
(13) In parenchyma		6.63	7.73	7.17	7.18	0.84	0.64
<i>(ii) In sclerenchyma</i>							
(14) In sclerenchyma		14.07	14.07	13.23	14.09	1.74	1.32

Note—1 Since the number of observations for the character (asterisked) was unequal, three pairs of C D (critical differences) would be necessary. They are given below—

Varieties	At 1%	At 5%
Between Saharanpur and Amritsar Paunda	22.8	17.3
Between Saharanpur and Peshawar Paunda	22.6	17.2
Between Peshawar and Amritsar Paunda	24.0	18.3

2 The magnitude of various features was given in the above table in divisions of eye-piece micrometer, the values of which were

- (a) For thickness of cell walls  
(Characters 6, 7, 8, 13 and 14) 1 division = 0.21  $\mu$
- (b) For area of vascular bundles  
(Characters 2, 3, 10 and 11) 1 sq division = 16.32 sq  $\mu$

3 (a) For counting the number of vascular bundles, the field of the microscope was taken as unit area the diameter of which was as follows

- (i) Character 1 1.7 mm  
(ii) Character 9 4.1 mm

(b) For the number of parenchymatous cells, the diameter of the field of microscope was taken as one of the sides of the square which measured

- (i) 0.68 mm for characters 4 and 5  
(ii) 1.53 mm for character 12

character only at 1% level. The Peshawar Paunda on the whole, therefore, occupied an intermediate position so far as the rind was concerned.

*The storage tissue*—In the central region, however, the Peshawar Paunda showed maximum magnitude for the 11th, 12th and 13th character and was intermediate for the 14th. It formed groups with the Saharanpur or Amritsar varieties or with both in all the characters except the 12th (Plate I, Figs 4–6) where it was significantly different from both of them even at 1% level, the difference in the size of the parenchymatous cells being clearly visible (Plate IV, Figs 7, 8, 9). The Saharanpur Paunda, in this region also, was the poorest in the development of characters 11, 12 and 13 (Plate III, Fig. 4). Only in the case of the 14th character it showed the highest value. In the 12th character alone it was significantly different from the other two varieties at 1% level. In other words the storage cells were the largest in this case (Plate IV, Fig. 8). At 5% level it differed significantly from Amritsar Paunda in the 14th character and from the Peshawar Paunda in the 13th character. The values for 11th, 12th and 13th characters for the Amritsar variety were intermediate and were not significantly different except for that of the 12th character (Plate III, Fig. 6). Its average value for the 14th character was the smallest and significantly different from the one for the Saharanpur Paunda at 5% level.

Summing up all the characters exhibited by the three varieties, Amritsar Paunda headed the list for five out of the ten characters and was significantly different in all of them. It was intermediate in four characters but differences in respect of three of them were not significant as compared to their maximum development. Only in one character it showed the least development but here too, the difference between this and the variety intermediate for this character (Table I) was not significant. Amritsar Paunda therefore, could be deemed to have secured eight top positions and two middle ones. For the Saharanpur Paunda, reverse was the case. It showed the least development for seven characters of which four were significantly different at 5% level. It had intermediate position for two characters but the difference between this and the lowest was significant only for one, at 5% level. In one character alone, it topped the list but it was not significantly different from the next variety. Thus it obtained for itself eight lowest and two intermediate positions. The Peshawar Paunda was intermediate as it had one distinctly highest and one definitely lowest position, the other eight being intermediate. The position of a variety in relation to the other two in respect of characters in which it was found to be significantly different is shown in Table II.

TABLE II  
Showing characters in which varieties are significantly different  
from one another

Characters \ Varieties	Salarnpur Paunda	Peshawar Paunda	Remarks
<i>Rind</i>			
Ieshawar Paunda	6 6 7 3		} Amritsar Paunda is the hardest of all in rind
Amritsar Paunda	3 4 5 6 7 8	3 4 5 6 8	
<i>Storage tissue</i>			
Peshawar Paunda	12 13		} Peshawar Paunda is the hardest of all in core
Amritsar Paunda	12 14	12	

Note — 1 Differences in characters italicized are significant at 5% level only, the rest at 1% level

- 2 Characters given above the horizontal line were better developed in the variety given on the left than in that given at the head of the column. Reverse was the case when a character appeared below the horizontal line

The full import of this consistent behaviour on the part of the varieties would be clear when all the characters were considered together

#### IV DISCUSSION OF RESULTS

A cane when cut across, reveals two component parts, viz, the rind and the core. The rind is formed by vascular bundles of various sizes, embedded in a parenchymatous matrix lignified to a varying degree. The character of the rind is, therefore, determined by the total area of vascular bundles, the size of parenchymatous cells and the thickness of cell-walls in both the tissues. The function that rind has to perform, naturally admits of little storage of sugar in its tissues. The core which forms the major portion of a cane, consists of thin-walled parenchymatous tissue in which isolated vascular bundles are scattered rather sparsely.

In a chewing cane, the rind has got to be differently constituted from that of a cane for general cultivation (*vide* Appendix). A soft rind for the latter is definitely a bad feature because of its inability to withstand the ravages of smaller animals like rodents and jackals, while in the former it is a valuable asset as the human jaw and teeth are comparatively a weak mechanism for biting and tearing purposes. At the same time it should not be so soft as to come off in chips when the cane is peeled. It should, therefore, be soft but coherent enough to be stripped from node to node. Besides, if it is thin and well demarcated also, the cane would be ideal so

far as one part of chewing process is concerned. A soft rinded cane need not present any serious obstacle in successful cultivation, because it being a garden crop, damage by animals can be reduced to a great extent by growing them in properly protected plots.

The core of a chewing cane is not fundamentally different from that of an ordinary one, meant for crushing (*vide* Appendix). Only it has to be much softer so that it can be pressed by human jaw and juice extracted from individual cells. It is, therefore, essential that the storage cells should be as large as possible with very thin walls. The vascular bundles should obviously be spaced far apart.

From these theoretical considerations, a cane possessing a thin and soft rind and yet adequately tough to be stripped clean from node to node, together with a soft core requiring the least effort to peel and crush it, would be ideally suited for chewing purpose.

Before discussing the relative merits of the three varieties as chewing canes, it would be necessary to eliminate the inequalities of numerical expression of various characters, so that their magnitudes could be compared to one another. Table III was compiled by expressing the magnitude

TABLE III  
Showing the magnitude of various characters as percentages of their respective totals

Characters	Varieties		
	Sahara pur Pau la	Peshwar lands	Amritsar lands
<i>Rind—Peripheral region—</i>			
A Vascular bundles			
(1) Number per unit area	30.0	31.0	39.0
(2) Size	35.8	29.7	34.5
(3) Total area occupied by them in unit area	32.2	27.5	40.2
B Number of Parenchymatous cells			
(4) In outer unit area	29.6	30.9	39.6
(5) In inner unit area	32.5	33.0	34.5
C Thickness of cell walls			
(i) Parenchyma			
(6) At 0.6 mm	30.2	33.2	36.6
(7) At 1.2 mm	29.6	35.3	35.1
(ii) Sclerenchyma			
(8) In sheath	31.3	30.4	38.0
<i>Storage tissue—Central region</i>			
A Vascular bundles			
(9) Number per unit area	35.0	36.2	28.8
(10) Size	27.2	33.1	39.7
(11) Total area occupied by them in a unit area	29.7	35.4	34.9
B Number of Parenchymatous cells			
(12) In unit area	28.7	37.8	33.5
C Thickness of cell walls			
(13) In parenchyma	30.8	35.9	33.3
(14) In sclerenchyma	35.4	33.3	31.3

of a character in a particular variety as percentage of its total for all the three varieties

From Table II given earlier, it would appear that even at 1% level, the Amritsar variety was significantly different from the Saharanpur Paunda in all the six characters of the rind and from the Peshawar Paunda in four of them. Further it maintained consistently the highest position (Text-Fig 1) in respect of the magnitude of almost all characters each of which made the rind hard to the extent of its development. It was, therefore, evident that the Amritsar variety had the hardest rind. In respect of the storage tissue, the Peshawar Paunda was harder than the Saharanpur variety, because the number and thickness of walls of the parenchymatous cells—two out of four characters pertaining to the core—were significantly greater in the former than in the latter. Although the lignification of sclerenchyma was higher in the Saharanpur variety than in the Peshawar Paunda, the difference between the two magnitudes was not found to be significant,

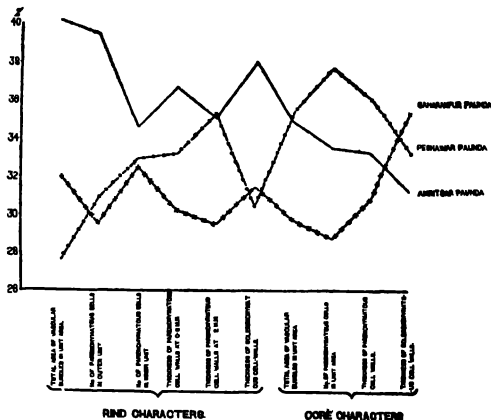


FIG 1 Anatomical Features of Chewing Caeop

and as such greater thickness of cell-walls in the former could not be regarded as having materially contributed much to make its core harder than that of the latter. As compared to the Amritsar variety also, the core of the Peshawar Paunda appeared to be hard because of the significantly greater number and therefore smaller size of parenchymatous cells per unit area.

Since the Saharanpur Paunda changed positions with Peshawar variety in respect of certain characters in rind and with the Amritsar variety in those of the core, it might not be the softest because it was just possible that the greater development of some of the characters might more than offset the total advantage given by the poorer development of the rest of the features in their respective spheres. If the characters in which they were not found to be significantly different, were left out in the final reckoning of hardness for the reason stated in the preceding para, one had only to examine whether hardness imparted to the rind by greater area of vascular bundles in the Saharanpur variety was so much as to nullify the total softness due to the poorer lignification of the parenchymatous matrix as revealed by the thickness of cell-walls at depths of 0.6 mm and 1.2 mm from the epidermis and the lesser number and therefore larger size of parenchymatous cells in the outer unit area. Similarly for the core, it would be necessary to find out whether the greater development of sclerenchyma had more than counter-balanced the effect of lesser number and therefore the larger size of the parenchymatous cells per unit area. A similar reckoning of hardness of the regions in question for the other two varieties, viz., Amritsar and Peshawar would be essential before any comparison could be made.

In assessing the combined effect of different characters which pulled in opposite directions, weightage given to any one of them on theoretical considerations would be more or less arbitrary as no direct experimental evidence could be obtained on this point. Since the total area of vascular bundles and the thickness of parenchymatous cell-walls are distinctively varietal characters and independent of each other, they have to be taken as of equal status. Whether the magnitude of the lignification of parenchymatous cells at each depth in the rind is independent of the other and, therefore, should get the same weightage as the vascular area, or each is the function of the other and therefore, the two being interdependent, can be best represented on 50-50 basis, has to be decided before evaluating the total hardness of the peripheral region in a cane.

If the thickness of parenchymatous cell-walls at 1.2 mm had shown the same ratio to that at 0.6 mm in all the three varieties, it could not have

been explained except on the basis of the former being dependent on the latter. But that is not so (Text-Fig. 2). That the lignification of cell-walls

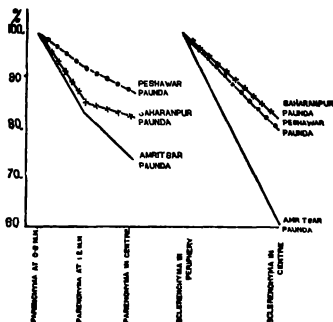


FIG. 2 Thickness of Cell Walls

in different parts of the cross-section of a cane was an independent character was further supported by the fact that in all the three varieties the thickness of parenchymatous cell-walls at 1.2 mm did not show the same relationship to that in the centre, and that the rate of its fall also was not the same for all. Observations on sclerenchyma confirmed this view (Text-Fig. 2).

On this basis, giving equal weightage to all the three characters, the Saharanpur Paunda was found to be four points less hard than Peshawar Paunda at 1% level (Table IV). At 5% level it gained one more point because of the lesser number of parenchymatous cells per unit area. Even if characters 6 and 7 were taken as components of one feature and not at par with the third character, each therefore, being eligible to only 50% weightage of the latter, the Saharanpur variety appeared to be just 0.3 point harder than the Peshawar Paunda at 1% level and one point less hard at 5% level because of the fourth character coming into the picture. So in 3 out of 4 computations the former scored over the latter as being less hard so far as the rind was concerned. In the core also, it was slightly (0.7 point) superior to the Amritsar cane at 5% and considerably (4.8) so at 1% level.

TABLE IV

Showing weightage of characters in which Saharanpur Paunda is significantly different from other varieties

Characters	Saharanpur Paunda	Peshawar Paunda	Saharanpur Paunda (+) or (-)	Remarks
(a) <i>In hand</i>				
At 1% level				
$3 + 6 + 7$	91.9	96.0	4.1	Saharanpur is softer than Peshawar Paunda
$3 + \frac{6+7}{2}$	82.0	61.0	+0.3	More or less equal
At 5% level				
$3 + 4 + 6 + 7$	121.5	126.9	-5.4	Saharanpur Paunda is softer than Peshawar Paunda
$3 + 4 + \frac{6+7}{2}$	91.6	92.6	-1.0	
		Amritsar Paunda		
(b) <i>In storage tissue</i>				
At 1% level				
$12 + 14$	64.1	64.8	-0.7	Saharanpur Paunda is softer than Amritsar Paunda
At 5% level	28.7	28.5	-4.8	

Note—Since greater magnitude means more of hardness, (+) indicates harder than and (-) 'softer than

In addition to its being the softest of all the three varieties, Saharanpur Paunda had another advantage. Due to the rapid fall in the lignification of the matrix, its rind was thinner and much better demarcated than that of the other two. In this case there was practically no further decrease in the magnitude of this feature (Text-Fig. 3) as one approached the storage tissue whereas in the other two varieties, such delineation was not found to exist, within 1.2 mm from the epidermis. Should it occur farther in the Saharanpur Paunda would at least retain the desirable feature of having a thin rind, which comes off easily and clean when stripped. The rind of the other two varieties due to greater and uniform thickening of the cell-walls of the matrix would obviously require more effort to peel.

From the foregoing discussion it would appear that of the three varieties, the Saharanpur Paunda was found to be the closest to the ideal chewing cane both in respect of the rind and the storage tissue. The Amritsar Paunda came next in respect of the latter region but was the farthest so far as the rind was concerned. The Peshawar variety occupied an intermediate position as regards the rind but was the hardest in the core.

It is interesting to note that the shape of parenchymatous cells was different in these varieties (Plate IV, Figs. 1-9). They were strongly oval

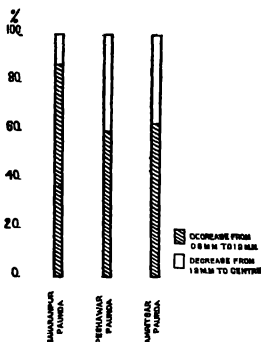


FIG. 3 Decrease in the Thickness of Parenchymatous Cell Walls

in the case of Amritsar Paunda, and less so in that of Saharanpur variety while in the Peshawar Paunda they were almost circular. The shape of these cells, however, did not appear to have any bearing on the suitability of a variety as a chewing cane.

#### V SUMMARY

1 The present paper tries to visualise from anatomical point of view an ideal chewing cane which besides having a soft core, possesses a thin and well defined rind that comes off clean with minimum effort when it is peeled.

2 The following characters which imparted hardness to the core and rind of the cane stalk were studied for three well known chewing-canes, viz, the Amritsar Paunda, the Saharanpur Paunda and the Peshawar Paunda.

(a) Total area under vascular bundles per unit area as calculated by adding the products of the radial and tangential axes of all the vascular bundles found in the field of the microscope. The product of the two axes of a vascular bundle in peripheral region bore a very high positive correlation of the order of 0.9546 with its area as determined by a planimeter.

(b) The number of parenchymatous cells per unit area, which obviously was inversely proportional to their size

(c) The thickness of cell-walls in the parenchyma at two depths from the epidermis, viz, 0.6 mm, 1.2 mm and in centre, and in the sclerenchyma, in periphery and centre

3 The Amritsar and Peshawar varieties were hardest in the rind and the core respectively because of the maximum development of all the characters in those regions

4 The rind of the Saharanpur variety was softer than that of the Peshawar Paunda, because the poorer lignification of the parenchymatous matrix in the former more than compensated for its greater area under vascular bundles whereas in the latter variety, the ground tissue was lignified so highly that it offset total softness accruing from lesser area of vascular bundles. Moreover, due to the rapid decrease in the thickness of cell-walls of the ground tissue, the rind was thin and well demarcated so that it peeled off clean with minimum effort

5 In the core, the Saharanpur Paunda had the smallest number of storage cells per unit area and, therefore, the largest in size, on the basis of which it was reckoned as softer than the Amritsar Paunda because in other characters, viz, the vascular area, the lignification of the parenchyma and sclerenchyma, it was not significantly different from that variety at 5% level

6 The Saharanpur Paunda was, therefore, closest to the ideal chewing cane and the respective positions of the three varieties might be graphically shown as below

Chewing cane	{ Rind—the Saharanpur—the Peshawar—the Amritsar
	{ Core—the Saharanpur—the Amritsar—the Peshawar

7 A chewing cane was found to be constituted on a pattern entirely different from that of a factory cane. The vascular bundles in the rind region of these varieties of chewing canes were found to be nearly half the size of those of the factory canes of the province. The total area under them in unit area was much higher in the latter than in the former. The cell-walls in both the tissues all over the cross-section were found to be much more highly lignified in the factory canes than in the chewing varieties

## VI ACKNOWLEDGEMENTS

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#### EXPLANATION OF PLATES

##### PLATE III

*T S of Stem of Chewing varieties of Sugarcane showing the Rind and Storage Tissues*

**Rind Tissue** Figs 1-3 Fig 1 Part of T S of rind of Saharanpur Paunda showing parenchymatous matrix formed by cells larger than those of the Amritsar variety (Fig 2), but with poorer lignification. The rind of the latter variety has greater number of vascular bundles than those of the Saharanpur and Peshawar Paundas (Figs 1 and 3), and so also the thickening of sclerenchymatous cell-walls resulting in great reduction of the lumen of cells (Magnification  $\times 80$ )

**Storage Tissue** Figs 4-6 Fig 4 Part of T S of the storage tissue of Saharanpur Paunda showing cells larger in size than those of the Peshawar Paunda (Fig 6). The Amritsar variety (Fig 5) occupies an intermediate position in this respect but has the smallest number of vascular bundles (Magnification  $\times 15$ )

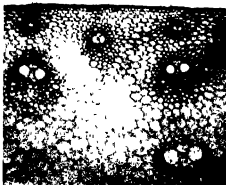
##### PLATE IV

*T S of Stem showing the Size of Parenchymatous Cells at Three Depths from the Epidermis*

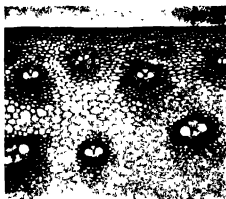
Figs 1, 2 and 3 at 0.6 mm, Figs 4, 5 and 6 at 1.2 mm and Figs 7, 8 and 9 at 2.4 mm. In Amritsar Paunda (Figs 1, 4) the cells forming the parenchymatous matrix of rind are the smallest and most highly lignified, whereas in the Saharanpur variety (Figs 2, 5) they are the largest in size with thinnest walls. The Peshawar Paunda (Figs 3 & 6) occupies an intermediate position being nearest to the Saharanpur Paunda.

In the storage tissue, however, the former has the smallest cells (Fig 9) followed by the Amritsar and Saharanpur varieties (Figs 7 and 8). The shape of the cells in the three varieties is also different (Magnification  $\times 200$ )

RIND TISSUE



1



2

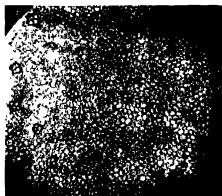


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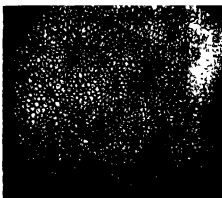
STORAGE TISSUE



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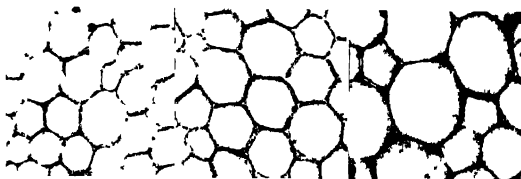


5



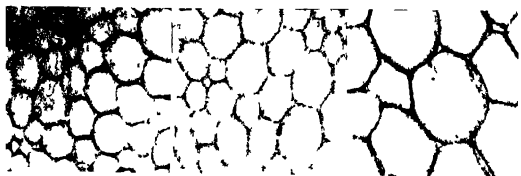
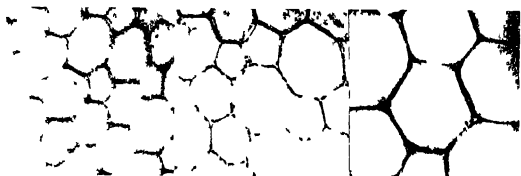
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*K I K r r Pr Int Act Sci I I NNI II IV*  
*nt S I S r nr*



1

4



3

1

## APPENDIX

## CHARACTERISTIC FEATURES OF A FACTORY CANE

A factory cane is characterised by a comparatively high fibre content, as distinct from that used for chewing purposes. A low fibred variety apart from presenting considerable mechanical difficulties in crushing would not be an economical proposition to mill because of the insufficient bagasse which is used as a fuel. Moreover, it should be hardy enough to withstand the vagaries of weather and other adverse circumstances so that it can be grown on an area commensurate with the needs of a factory.

These fundamental differences between the two categories of cane varieties are broad-based on their anatomical make up (Table I). In the case of a chewing cane, the rind is soft and lignification of tissues both parenchymatous and sclerenchymatous all over the cross-section of a cane, poor, while a factory cane has to possess a fairly hard rind and moderately lignified tissues.

While in the rind the number of vascular bundles per unit area in the factory canes was significantly less than that in the chewing varieties except the Saharanpur Paunda, their size and the total area occupied by them in the former group were nearly twice as much as were found in the chewing canes. In the case of the storage tissue although the number of and total area occupied by vascular bundles were not directly comparable because of the difference in the size of unit area taken for the two types of cane varieties, there did not appear to be any appreciable difference between the two groups of the cane varieties so far as these characters were concerned. This view appeared to be supported by the average size of individual vascular bundles which was more or less the same in the two groups, because the difference in its size in various varieties was not found to be significant even at 1% level.

The difference in the anatomical make-up was much more categorical when the thickness of cell-walls of the parenchymatous and sclerenchymatous tissues, was considered. In Co 210 and Co 213 the thickness of the cell-walls in the parenchymatous matrix at both the depths in the peripheral region was nearly three times and in the central region approximately twice of the general mean for the chewing canes. As regards the sclerenchyma forming the vascular sheaths in the rind, the lignification of cell-walls in Co 210 and Co 213 was nearly three and two times respectively of that found in the chewing varieties. In the central region, however, the difference as regards this feature was not so pronounced. Still the thickness of these cell-walls was nearly 25% as much more in these varieties

TABLE I

Showing anatomical features of factory and chewing sugarcane varieties

Character	Chewing canes				Factory canes			Critical diff bet the two groups at 1% level
	Amritsar	Shahranpur	Peshawar	General mean	Co 210	Co 213	Co 313	
<i>Rind Peripheral region—</i>								
<b>A. Vascular bundles</b>								
(1) Number per unit area	12.93	9.94	10.25	11.04	9.17	8.87	8.96	1.028
(2) Size	170.96	177.73	147.41	165.7	40.50	31.637	367.66	17.970
(3) Total area occupied by them in unit area	2207.8	1767.4	1511.0	1828.8	3708	2734	3297	
<b>B. Thickness of cell walls</b>								
(4) Parenchyma at 0.6 mm	0.78	8.00	8.81	8.94	23.13	23.31	11.56	0.984
(5) Parenchyma at 1.2 mm	8.10	0.81	8.16	7.69	23.69	21.81	11.56	1.151
(6) Sclerenchyma	21.06	18.19	17.08	19.24	54.50	39.31	29.94	2.396
<i>Storage tissue Central Region—</i>								
<b>A. Vascular bundles</b>								
(7) Number per unit area	9.22	11.22	11.60	10.67	3.44	3.73	3.14	
(8) Size	401.87	274.61	334.74	337.1	359.59	306.17	362.63	46.597
(9) Total area occupied by them per unit area	3628.2	3089.4	3664.7	3465.4	1237	1142	1139	
<b>B. Thickness of cell walls</b>								
(10) Parenchyma	7.17	6.63	7.75	7.18	13.94	13.08	13.44	1.375
(11) Sclerenchyma	13.23	14.96	14.08	14.9	18.36	17.50	21.19	2.320

Note—1 The magnitude of various characters was expressed in terms of the divisions of the eye piece micrometer the values of which were

(a) Thickness of cell walls 1 division = 0.21  $\mu$

(b) Size and total area of vascular bundles in

(i) rind 1 sq division = 14.4 sq  $\mu$  For factory canes

(ii) Storage tissue 1 sq — 15.2 sq  $\mu$

(iii) both the regions 1 sq 16.3 sq  $\mu$  For chewing canes

2 For counting the number of vascular bundles the field of the microscope was taken as unit area the diameter of which was as follows

(i) 1.7 mm for both the regions of factory canes and rind portion of the chewing varieties

(ii) 4.1 mm for the central portion of the latter

3 It would therefore appear that the magnitude of the features under discussion were more or less directly comparable in both the types of cane varieties except the number of and total area occupied by vascular bundles per unit area in the storage tissue where the diameters of unit area happened to be different

4 Critical difference for characters 7 and 9 could not be calculated for the reason given above and for the character 3 because the total area was arrived at by two different methods

than that found in Paunda varieties Co 313 which was the softest of all the varieties given out so far, for general cultivation as a factory cane, was found to have nearly 50% more highly lignified cell-walls in both the tissues all over the cross-section. It was harder than the hardest of the chewing

varieties, namely the Amritsar Paunda. All the three varieties were significantly different from the chewing canes at 1% level in respect of the lignification of both the tissues.

It would therefore appear that cane varieties in order to be useful for different purposes have got to be constituted on an entirely different basis. The work on milling features of sugarcane varieties is being reported separately.

# THE TAPIOCA PLANT AND METHODS FOR EVOLVING IMPROVED STRAINS FOR CULTIVATION\*

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CONTENTS		PAGE
I	INTRODUCTION	32
II	THE TAPIOCA PLANT ..	34
	Anatomy of Stem	37
	Anatomy of tubers	39
III	VARIETIES UNDER CULTIVATION IN TRAVANCORE	40
	Classification	42
IV	CULTIVATION OF TAPIOCA	43
V	GENETICAL WORK ON TAPIOCA	44
	1 Intervarietal hybridisation	44
	2 Interspecific hybridisation	46
	3 Back crossing	49
	4 Evolution of polyploid forms	50
	5 Evolution of triploids	53
	6 Evolution of pure strains of Tapioca	54
VI	CHEMICAL COMPOSITION OF TUBERS	56
VII	SUMMARY	57
	Literature cited	58
	Explanation of Text figures and Plates	59

## I INTRODUCTION

TAPIOCA or the Cassava plant (*Manihot utilisima*, Pohl) belongs to the milkweed family (Euphorbiaceæ). It is a native of Brazil, South America. In the botanical literature of the last century the plant is described as *Janipha manihot*, Kth or *Jatropha manihot*, Linn. It was introduced in this country more than a century ago. Drury in his "Useful Plants of India" published in 1858 has recorded that tapioca was then under extensive cultivation in Travancore. The Travancore State Manual has

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\* A Monogram on the work done in the Department of Research University of Travancore during 1940-47

stated ' that the popularity of this crop plant is specially due to the exertions of His Highness Sri Visakhram Thirunal Maharaja ' (1837-1885) Burkill (1904) states that early Portuguese settlers introduced tapioca to Goa as well as to Africa Macmillan (1925) records that Tapioca was introduced into Ceylon and India by the Portuguese in the 17th century

The success of Tapioca as a major crop has in large measure been due to the particularly favourable climatic and soil conditions of Travancore It is also likely that the growth habit and high yield of this plant ensured its popularity with the ryot It is a hardy plant thriving even in the most barren soil Little or no care is necessary for it after planting and the yield per acre is so high as to give for the ryot a good return for his labours In pre-war days a pound of fresh tapioca tubers did not cost more than two pies It was thus within easy reach of the poor and became practically the poor man's food in Travancore Tapioca cultivation in consequence steadily increased so that to-day it is second only to paddy as a major crop in this State

Owing however, to the primitive methods of cultivation employed by the ryot and the poor quality of the varieties used for cultivation, the average yield here does not exceed two tons of tubers per acre at present In Java, West Indies and other countries of the Far East where improved strains of tapioca are used for cultivation, the average recorded yield for this crop ranges from 10-15 tons per acre It should therefore be possible substantially to increase the yield of this crop by enabling the ryot to have better varieties of tapioca and by introducing improved methods of cultivation

During the war when rice imports from Burma were cut off, tapioca has been a boon to Travancore It saved the country from famine and its food value has received greater attention since then Tapioca has also assumed importance in recent years as its starch is in great demand as a suitable sizing material in textile industry There is no doubt, therefore, that the cultivation of this crop will receive greater attention in future The following account of the applications of modern genetical methods for evolving improved strains of tapioca undertaken at the Tapioca Research Farm\* in the Department of Research, University of Travancore, is therefore presented with a view to stimulating interest in this crop plant

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\* This Farm is maintained from the Pattabhirama Iyer Endowment Funds donated at the rate of Rs 1,000 per mensem by Sachuvottama Sir C P Ramaswamy Iyer, Dewan Vic-Chancellor

## II. THE TAPIOCA PLANT

Tapioca, known as '*maracheeni*' or '*kappacheeni*' in Malayalam is a crop plant cultivated in all dry soils in Travancore. Within a week after planting, two or three buds sprout from the nodes of the seed canes and grow up as erect branches. These stems branch repeatedly in threes and run into several such grades in an apparently trichotomous mode of branching (Fig. 1). While this type of branching with a spreadings shoot



FIG. 1. The Tapioca Plant

system is characteristic of all flowering varieties, it is significant that non-flowering varieties seldom branch, growing as erect, tall plants, reaching a height of 6-8 feet. The colour of the stem varies with varieties. It may be green, grey, pink, dark-brown or purple. Leaves stipulate, long petioled, palmate, divided nearly to their base into 5-7 lanceolate, entire lobes dark green above and glaucous beneath. Midrib prominent below and usually of the same colour as the petiole. Petiole long, inserted obliquely on the stem and arranged in a  $2/5$  spiral. Stipules thin, dissected, pointed, greenish white, occasionally with a reddish base falling off just after the leaf has fully spread. On an average about 15 leaves will be present on the terminal region of the branch. Leaves become mature and fall within about six weeks, leaving prominent nodal protuberances on the stem. These nodal swellings are surmounted by circular leaf-scars with an obliquely transverse knife-edge like extension on each side formed by the stipular scars. The stem thus presents a rugged exterior with these close-set and spirally arranged swellings. Cork formation commences early on the stem developing a scaly skin which can be easily peeled off.

Roots grow from the cut-end of the seed cane within a few days after planting. They are long, slender and white, spreading in the soil more or less horizontally about 3-5 inches below the surface. As the plant grows



FIG. 2. Young Plant growing from a cutting

older, some of these roots become tuberous while the majority of them continue to be thin and function as absorbing organs. Both absorbing and tuberous roots are spreading in habit so that hardly any root lies deeper than 8-10 inches in the soil. As the tuberous roots begin to store starch in them they gradually increase in thickness, developing when mature, a skin as in the stem, a rind and the starchy inner portion with a central strand of conducting tissue. In most varieties growth of the tubers is completed in about eight months.

The plants flower in about six months. The flowers are borne in terminal panicles. The first panicles usually appear at the junction of branches of the second grade (Fig 3). Flowers unisexual, protogynous.

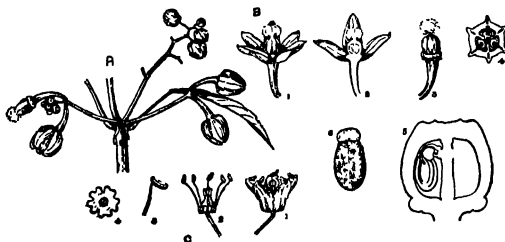


FIG. 3. Flowers and Fruit of *Tapioea*

Male flowers smaller than the female and usually in terminal clusters. Perianth cup-shaped, with five imbricate lobes enclosing a 10-lobed glandular disc. Stamens 10, springing from the base of the perianth and curving out through the lobes of the disc. In the open flower the anthers are arranged in two levels; five small with shorter filaments curved inwards and five large with spreading filaments. The small stamens are opposite the perianth lobes while the large ones alternate these lobes. Anthers dorsifixed and dehisce by longitudinal slits. Pollen grains are large, spherical and few in each sac. In the centre of the disc a pistillode is represented by a small conical projection. The female flowers are developed earlier than the male flowers of the panicle and are fewer in number. The perianth of the female flower is deeply 5-lobed and with purple border. Pistil has a basal ring. Ovary trilocular, spherical with a capitate 3-lobed

stigma Ovule one in each loculus, pendulous, anatropous with ventral raphe and micropyle directed upwards and outwards At the tip of the outer integument a soft tissue, the caruncle, is formed which caps the ovule There is also an obturator mechanism which is a peg-like process formed from the placenta This grows towards the ovule, curves round the caruncle and enters the nucellus through the micropyle (Fig 3 B 5) It is composed of thin-walled, elongated cells with rich contents The pollen-tube in its passage to the embryosac directs its course through the obturator This interesting mechanism thus serves as a short-cut to the micropyle besides being a nutritive tissue for the pollen tube as suggested by Strasburger (1921) After fertilisation the obturator disappears

Fruit globular, about one inch in diameter with six thin, narrow wings Capsule hard, splitting into three cocci each with a seed Seeds elliptical, black, grey or mottled, shining, resembling a castor seed The seed-coat is thick, hard and polished Inside the seed coat there is the endosperm which is massive enclosing the embryo The two leaf-like cotyledons of the embryo are pressed against each other by the endosperm The radicle of the primary axis is directed towards the micropyle

During germination the radicle pushes itself out through the micropyle and develops branch roots at its tip The hypocotyl is curved and by its further elongation the cotyledons are pulled out of the seed (Fig 4) They then expand, become green and behave like foliage leaves

#### *Anatomy of the Stem*

(a) *Macroscopical*—The stem is light, breaks easily and has a thin skin which gets easily peeled off exposing a green soft tissue inside A cut-end of the stem shows four prominent parts a central whitish pith, a broad ring of wood, a greenish bast and a brown skin The pith is a soft tissue about a centimeter in diameter in a young stem of normal thickness It is pentangular with rounded corners This tissue is enveloped by the wood The radial arrangement of the cells of the wood are well marked out on the cut surface Pores though few are conspicuous and regularly arranged Milky latex with a faint yellow colour is seen to exude in a ring in the bast and in small drops at the protoxylem region on the cut-end of a fresh stem The skin is dry and papery It breaks off in narrow flakes and consequently the surface of the stem is rarely smooth Lenticels are few The rind as well as the skin can be easily removed from the stem (Fig 5)

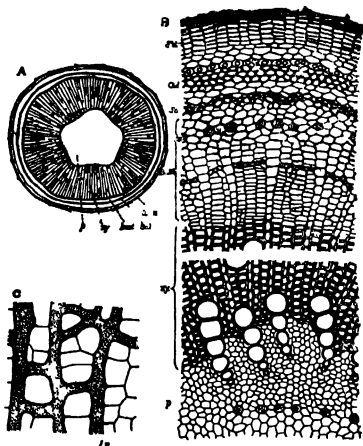


FIG 5 Cross Section of Stem

A Cut end of the stem seen under a lens B Tissues seen under the microscope  
C Latex vessels

(b) *Microscopical*—The skin is the cork tissue developed by a phellogen originating near the epidermis in young stems. It is composed of 8–10 layers of regularly arranged prismatic cells (Fig 5 B). More than half of these layers on the outside develop thick walls in their cells while the inner layers usually remain thin walled. The phellogen continues to be active producing newer layers of cork cells as older peripheral layers get peeled off in thin flakes. The cells of the cortex are more or less rounded in outline and have chloroplasts in them presenting a green colour to this tissue. Two or three layers on the inside of the cortex are transformed into collenchyma (Fig 5, 'col'). There is a layer of cells with inclusions of tetrahedral crystals just on the outside of this tissue. Cortex is limited on the inside by a ring of sclerenchyma of two or three layers of thick-

walled cells (Fig 5, 'sc') The fibrous nature of the rind is due to this ring The tissue inside this ring is the bast In shape as well as arrangement of cells the bast presents a complicated structure The inner layers of this tissue have radially arranged cells developed by the cambium The primary phloem is seen as disorganised groups of cells (usually five) in the outer region Latex vessels (lv in Fig 5) are present just on the border of the secondary phloem These are branching and anastomosing tubes, extending vertically and arranged only in one layer (Fig 5, C) The wall of the latex vessel is thin and its contents granular Between the bast and wood is the delicate ring of cambium It is composed of thin-walled cells and have their broad side along the tangential plane The bulk of the wood (Fig 5, B 'xy') is composed of fibres and medullary rays with scanty development of vessels The medullary ray cells are thin-walled, radially elongated and are rich in starch grains There may be two or only one such row of cells constituting a ray These rays become broader as they pass through the bast The fibres are only moderately thick walled with the result that the wood is soft and light The vessels though few in number have wide lumen and are conspicuous in the wood The primary xylem, usually five in number and characterised by radial series of large vessels are seen projecting into the pith at the inner extremity of the wood Pith is a soft tissue of thin-walled polyhedral cells (Fig 5, p) The outer layers of it are smaller and their walls thicker than those of the central tissue These cells are also seen to store starch in them A few latex vessels are developed in this tissue just on the inside of the proloxylen groups The cells towards the centre are large, polyhedral and thin-walled and have little or no starch grains in them

#### *Anatomy of the Tubers*

The anatomy of the tuber is essentially similar to that of the stem except for the structure of the central vascular strand and of the 'flesh' The young root has the typical dicotyledonous structure with 4-6 exarch, radial bundles Endodermis is well developed Tuber development in it is initiated by the cambium ring This ring produces towards its inside, almost exclusively, thin-walled cells which act as store houses for starch grains While these cells have thus the same origin as wood cells following more or less the same arrangement as well, thick-walled fibres are seldom developed with the result that the soft 'flesh' of the tuber is formed This tissue is thus secondary xylem specially formed for storage As in the stem it is enveloped by a rind, with almost similar structure The skin which envelopes the rind has also the same origin and structure as in the stem These details are shown in Fig 6

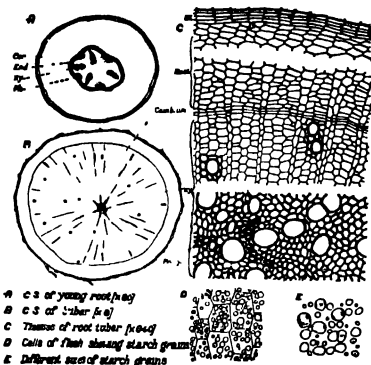


FIG 6 Cross Section of Tuber

### III VARIETIES UNDER CULTIVATION IN TRAVANCORE

Two principal forms of tapioca, the 'bitter' variety (*Manihot utilisima*) and the 'sweet' variety (*M. alpi*, Plon) were recognised in earlier times in America. But specific differences between them being so slight and inconspicuous and the forms which have developed by centuries of cultivation so numerous and intergrading that this grouping is no longer followed. In Travancore 'aviyan' and 'maravan' both belonging to *M. utilisima* have been taken as two chief groups, the tubers of the former boiling easily while the latter required more time for boiling to remove the deleterious juice. The following are some of the chief varieties, the vernacular names signifying broadly the characters either of the stem or of the tubers.

*Vella maracheeni*, *Chenkompan*, *Pacha aviyan*, *Anai maravan*, *Malai elavan*, *Ariyan Rottivella*, and *Kanchavu ariyan*.

With a view to ascertaining the nature and extent of variation in the indigenous varieties, an extensive collection was undertaken by the Econo-

mic Botany Section of the Department of Research Seventy-three so-called varieties were collected from different parts of Travancore Two varieties were brought from Mysore by the author and one Java variety was obtained from Bangalore Of these 76 varieties 20 did not survive The remaining 56 varieties were studied with reference to their morphological characters according to the scheme given below

*Scheme for description of the Plants*

- Plot No
- Number of the plant
- Date of planting
- Growth Habit
  - (a) Erect or Spreading
  - (b) Branched or not
- Colour of
  - (a) Old Stem
  - (b) Young Stem
  - (c) Leaf-bearing portion
- Colour of
  - (a) Petiole
  - (b) Stipule
  - (c) Leaf
  - (d) Budleaf
- Date of Flowering
- Colour of
  - (a) Male Flower
  - (b) Female Flower
  - (c) Fruit
  - (d) Fruit wings
- Tuber—Colour of
  - (a) Skin
  - (b) Rind
  - (c) Flesh
- Date of Harvesting
- Number of Tubers per plant
- Total weight of tubers
- Percentage of starch
- Percentage of HCN

*Method of Classification*

Greenstreet and Lambourne (1933) have classified tapioca into two groups with reference to the colour of the cortex being either light green or dark green. As growth habit is a more permanent varietal character in tapioca a revised scheme of classification has been made by the author as given below. Careful observation of their external features has enabled an accurate estimate of the extent of variability of the characters possessed by these plants. Three more or less permanent varietal characters were taken as the basis for a classification of the recorded varieties. These are (1) colour of stem, petiole and tuber, (2) growth habit and (3) flowering or sterile.

The following scheme of classification has accordingly been evolved, reference to the registered varieties pertaining to each group being shown against each.

*Classification of Indigenous Varieties of Tapioca***GROUP I—*Erect, Tall-Branching—***

*Type 1—*Mature stem grey, petiole red, tuber skin white

A—Budleaf green—*Nos* 10, 12, 46

B—Budleaf pink—*No* 8

C—Budleaf pink, male sterile—*Nos* 4, 5, 36, 54, 73

*Type 2—*Mature stem grey with black tinge, young stem with red streaks. Petiole red, budleaf green, tuber skin brown, rind, red—*Nos* 52, 63

*Type 3—*Mature stem brown, tuber brown

A—Petiole red, budleaf pink, tuber rind reddish—*Nos* 18, 47

B—Petiole red, budleaf pink, tuber rind white—*Nos* 7, 38

C—Petiole red, budleaf green, tuber rind cream—*Nos* 6, 28

D—Petiole deep red, budleaf pink, tuber skin reddish brown, rind white—*No* 9

E—Petiole yellow along lower and red along upper side, budleaf green, tuber rind cream—*No* 24

*Type 4—*Mature stem deep brown. Branches stunted and bushy. Petiole short yellow with reddish base. Tuber brown, long and with elongated stalk. Budleaf green. Male sterile—*Nos* 30, 75

**GROUP II—*Erect Low-Branching—***

*Type 1—*Mature stem grey, tuber skin white, nodal swellings prominent close set

*The Tapioca Plant & Methods for Evolving Improved Strains* 43

A—Petiole red, budleaf pink—Nos 3, 23, 39, 43, 67

B—Petiole red, budleaf green—Nos 1, 15, 40, 41, 48, 70, 76

C—Petiole green, with reddish middle and pink base, budleaf green, male flowers sterile—No 32

Type 2—Mature stem grey with black tinge young stem green, petiole all green, budleaf green, tuber white—Nos 21, 26

Type 3—Mature stem grey, young stem yellowish green, petiole yellowish green

A—Budleaf green—Nos 13, 35

B—Budleaf pink—No 45

Type 4—Mature stem brown petiole red, budleaf pink, tuber brown—Nos 11, 42, 49, 74

Type 5—Mature stem greyish black petiole red, tuber skin white

A—Budleaf pink—Nos 17, 27, 65

B—Budleaf green—Nos 56, 66, 68

GROUP III—*Spreading, Profusely Branching*

Type 1—Mature stem reddish brown, petiole yellow, tuber brown,

A—Budleaf pink—No 61

B—Budleaf green, Male sterile—No 62

Type 2 Mature stem blackish brown, petiole yellow, budleaf green, tuber brown, rind and flesh yellow—Nos 29, 51

GROUP IV *Tall, erect, rarely branching, Non flowering Stem greyish black, wood yellowish, tuber brown*

Type 1—Petiole black-red, budleaf green—Nos 22, 37

Type 2—Petiole red on the upper and yellow on lower side with base red Leaf-base prominent—No 57

IV CULTIVATION OF TAPIOCA

Travancore is an agricultural country, nearly half of the population depending almost exclusively upon land for their livelihood. Of its total area of 7,662 sq miles, the wet land along the west coast and the hills and forest along the east, take up nearly half this area so that only about 3,500 sq miles of land are available for cultivation in Travancore. As this area is fragmented into small holdings, large-scale cultivation of extensive areas as in other countries is not possible here nor can a uniform method of cultivation be followed for any crop. Roughly about a fourth of this area or about 5,00,000 acres of land is now used for the cultivation of tapioca.

Tapioca plant can withstand long-continued and extreme drought but the seed canes need abundant moisture to sprout. The cultivation of this crop in Travancore is thus regulated with reference to the two monsoon seasons. In central and north Travancore the crop known as '*karkadaka kappa*' is harvested in the month of Karkadagam (July-August) and the land is prepared for fresh planting by October when the N E monsoon rains render suitable conditions for seed canes to sprout well. In south Travancore, however, cultivation commences in April or May depending on the S W monsoon for its water supply and the crop is harvested in January or February. As December to April are the dry months of the year, the central Travancore crop has to pass through the entire dry season. Although this is a serious disadvantage, the fact that this crop gets the full benefit of the entire S W monsoon rains during the period of maturity may perhaps be an advantage in tuber development. This however has to be ascertained by comparative yield trials.

The ground is prepared for planting by ploughing followed by a harrowing to smooth off rough edges of the field. The seed canes are cut in pieces 6-8 inches in length, and the cuttings are planted erect, usually two in each pit. The pits are usually  $2\frac{1}{2}$  3 feet apart. Cattle dung and ash are the only manures, employed either in pits before planting or as basic dressing before ploughing the field. In some places planting is on small mounds 3 feet apart with three cuttings in each mound. Except for a weeding operation when the ground is carefully loosened after about a month, little or no care is given to the crop. Two or three buds grow from each seed-cane. These grow up and may become branched, developing leaves rapidly on the elongating axis. As the plant becomes mature all but a few stunted leaves at the top are shed. Harvesting is done at this stage either by digging up the tubers if the soil is hard or pulling up the plant with the tubers. The yield of tapioca is as variable as that of other cultivated crops, depending on the nature and fertility of the soil as well as the quality of the seed canes. On an average  $2-2\frac{1}{2}$  tons per acre is considered a fairly good return by the ryot.

## V GENETICAL WORK ON TAPIOCA

### 1 Hybridisation—Intervarietal

Tapioca is essentially a vegetatively propagated plant, although most of its varieties flower profusely and set seeds. In order to undertake genetical studies on this plant, the conditions necessary for the germination of its seeds had first to be ascertained. Seeds of three local varieties were collected and extensive germination trials were made in the Botany

**Department** In the first set of experiments no seed germinated even after three months. The seeds were then subjected to certain pre-treatments. In one set of experiments the seeds were soaked for varying periods before sowing in pots. This too did not yield favourable results. In another set the testa at the hilum region of the seed was rasped before planting in seed-pans. Out of thirty seeds thus treated, one seed germinated. Soaking the seeds for 2-3 days maintaining a constant temperature of 35-37° C was found more successful. Petridishes with moist sand and 4-6 seeds in each were left for one week in an incubator at a constant temperature of 37° C. The seeds responded to this treatment well and within a week almost all seeds sprouted. At this stage the seeds were transferred to the field. On an average about 80% of seeds thus transplanted grew up into vigorous plants.

Having thus evolved a technique for hastening germination, hybridisation was taken up on eight varieties of tapioca grown in a small plot of land behind the University Office. Tapioca flowers being unisexual, cross-pollination is easy. Female flowers to be crossed were covered over by muslin bags early in the morning of the day they open. Usually these flowers open by about noon. Mature male flowers (of plants selected as male parents) are then collected in a dish with a little water just before these flowers open. Pollination of the 'bagged' flowers is then effected by carefully opening the muslin bag and rubbing the anthers of a male flower on the stigma of the female flowers. The bag is again tied round the female flower and the ovary is left undisturbed to ripen into fruit. Usually the fruits ripen from 80-100 days after pollination. The hybrid seeds obtained by reciprocal crosses of five varieties of tapioca were grown and the plants were studied with reference to their cytology and genetics by one of my research students, Mr T J Koshy. Meanwhile Mr A Abraham, Economic Botanist, also undertook hybridisation work at Kayamkulam. Over 1,300 hybrid seeds belonging to 122 crosses were obtained by him. With the organisation of a Research Farm for tapioca at Trivandrum further work on this crop plant became possible. Over 700 plants from these hybrid seeds were planted for yield trials in April 1945. The weight of the tubers of each hybrid plant was recorded during harvest and a selection of 173 plants was made for further work. Of these, 91 plants with ten replications of each were planted in a plot 220 ft. × 32 ft., 41 plants with five replications of each in a plot 120 ft. × 14 ft., and another 41 plants each with five replications, in a third plot measuring 120 ft. by 14 ft. Plantings in these three plots have been in Balanced Incomplete Block experiments designed by the Statistical Department of

the University. On the basis of yield data 23 varieties have now been selected for the third year's cultivation, the average yield of each selected variety being over 10 lbs.\* per plant. The outstanding morphological characters of these plants together with their tuber-weights in the first and second year's cultivation are shown in Table I

Number of Hybrid	Parents	Average tuber wt per plant		Morphological characters
		1 year	II year	
		lbs oz	lbs oz	* F
114	28 x 27	5 5	9 9	B ls F
117	28 x 29	7 12	10 0	T E (F)
94	29 x 35	2 14	10 9	B ls F
96	do	3 11	13 7	B ls (F)
99	do	3 0	10 7	B ls F
177	do	3 12	10 7	B ls F
195	do	7 4	12 6	B ls F
228	29 x 45	4 2	12 1	B ls F
239	do	2 6	12 2	B ls F
105	29 x 47	3 11	19 5	B ls F
106	do	7 7	10 8	T E (F)
107	do	6 12	10 0	T E (F)
108	do	6 10	12 8	B ls F
369	34 x 29	4 4	10 1	B ls F
686	do	6 14	11 3	B ls F
808	do	4 1	10 0	T E F
874	38 x 29	9 2	10 5	B ls F
895	do	11 0	12 6	B ls F
896	do	7 0	13 5	B ls F
408	do	4 4	12 6	B ls F
894	do	4 9	10 0	T E F
368	38 x 45	4 0	10 8	T E (F)
479	38 x 63	3 4	10 0	B ls F

\* B-Branching; E-Erect; T-Tall, ls-low-spreading; hs-high-spreading; F-Flowering; (F)-Non-flowering.

By continued selection work on these lines it would be possible to make a final selection of a few which are markedly superior to the remaining in yield. These selected varieties of hybrids will be multiplied and made available to the ryot for extensive cultivation.

## 2. Interspecific Hybridisation

Tapioca has a closely related plant *Manihot glaziovii* (carea-rubber plant) in Travancore. It is also a native of tropical America having been introduced in this country about the same time as tapioca. Before Hevea

\* As some 4,500 plants are grown per acre, an average yield of 10 lbs. per plant corresponds to a yield of 20 tons per acre.

rubber plants became more popular for the rubber plantations of Travancore, Ceara-rubber was under cultivation here, though on a limited scale. It is a medium-sized, spreading, quick-growing tree 40-50 feet high. Inter-specific hybridisation between tapioca and Ceara was undertaken by Mr A. Abraham at Kayamkulam with a view to introducing, if possible, favourable genes into tapioca. Cross-pollination with Ceara-rubber plant as female parent was not successful while from 391 crosses with 13 varieties of tapioca as female parent, 8 seeds were obtained. Three of them germinated and grew up as tall, robust plants. Of these, hybrid 34 × R\* exhibited the phenomenon of gigantism more markedly than the other two, 63 × R and 29 × R. All of them were different from either the tapioca or the rubber parent. Hybrid 34 × R was pulled out when 14 months old. Tubers of medium thickness, but longer and more numerous were seen developed on it. The other two plants are still growing in the Farm with a Ceara-rubber plant nearby. These are shown in the adjoining photograph (Fig. 7).

The outstanding morphological characters of hybrid 63 × R are shown in Table II along with the characters of its parents for comparison.

TABLE II

Morphology	Parent No. 63	Hybrid 63 × R	<i>M. glauca</i>
Growth habit	Erect tall branching	Erect very tall branching at 10	A tall tree
Stem	Grey with nodal swellings	Reddish brown. No nodal swellings	Reddish brown. No nodal swellings
Petiole	Purple	Purple	Green
Leaf	7 lobed middle broad	3-7 lobed middle broad	5-7 lobed distal broad
Age at flowering	4-6 months	12-14 months	12-14 months
Male flower	Pale green	Pink	Green
Fruit	Dark green	Green with violet tinge at base. Pedicel violet	Deep green
Fruit wings	Prominent	Not prominent violet tinged	No wings
Tuber skin	Brown	Yellowish brown	Dark brown
"    "    "    "	Pink	Pale pink	Cream

As there is marked variation in the shape of the leaves, a mature leaf from each of these plants is shown in Fig. 8 A.

During last year this hybridisation work was continued and 38 plants were obtained. They have also grown up into as robust plants as the first three plants. The female parents of these hybrids with the number of plants obtained in each cross are shown below.

\* Ceara-rubber plant as male parent

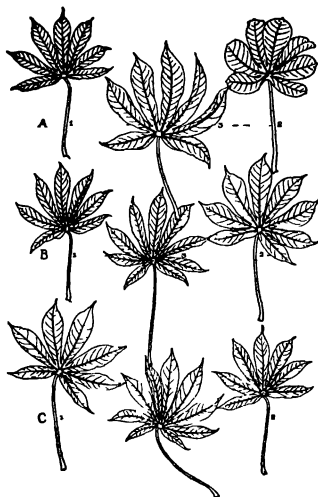


FIG 8 Variation in leaves of A (1) Tapoca No 63 (2) Rubber plant and (3) Inter specific Hybrid B (1) No 27 tapoca (2)  $34 \times R$  Hybrid (3)  $74 R \times 27 C$  C (1) Tetraploid  $3/10$  (2) Diploid No 63 (3) Triploid type

$29 \times R$	9 plants	
$32 \times R$	2	(Male sterile)
$36 \times R$	5	(Male sterile)
$38 \times R$	5	
$48 \times R$	4	
$51 \times R$	1	
$61 \times R$	4	
$73 \times R$	2	
$74 \times R$	6	

Although all these plants have also flowered, plants derived from female parents Nos 32 and 36 have only sterile male flowers. In their external appearance these hybrid plants are markedly different from either the tapioca plants or the ceara rubber plant. The nodal protuberances characteristic of tapioca stems are absent in them although leaf-scars are well marked out. The colour of the stem is usually seen to approximate more closely the female tapioca parent than the stem of the rubber plant. The first few leaves of the plant resemble the rubber plant more closely than those of tapioca. It is significant however, that as these plants grow up, the leaves gradually become so transformed as to resemble more closely the tapioca type. Fig 9 illustrates this aspect of hybrid growth very clearly. It is a photographic reproduction of three plants, a seedling of ceara-rubber (A), a hybrid seedling  $74 \times R$ , (B) and a young hybrid plant grown from a cutting of  $34 \times R$  (C). The leaves of the ceara-rubber plant can easily be recognised by the 5-lobed obovate, close set leaves. In the hybrid plant B, the lobes of the first formed leaves are obovate, but have acute tip. After forming about a dozen such leaves, they become narrower as seen in the upper leaves of hybrid B, approximating the leaves of tapioca. In the mature hybrid plant the leaves are closely similar to the tapioca type as seen in plant C, which is derived from a cutting of the hybrid.

Further work on these has been directed on (1) back-crossing, and (2) self-pollination.

### 3 Back-crossing

Using 29 R as male parent on variety No 29 four seeds were obtained. Hybrid 34 R was back-crossed with two varieties similar to 34 (Nos 27 and 63). The seeds obtained by this have been planted and two plants—one belonging to each parent—have grown up. Fig 8 B shows a typical leaf of No 27 (1) of hybrid 34 R (2) and of hybrid  $34 R \times 27$  (3).

Outstanding morphological characters of these are shown in Table III.

TABLE III

Morphology	Hybrid 34 R (Male)	No 27 (Female)	Hybrid 34 R $\times$ 27
Growth habit	Tall spreading in umbrella form	Low branching	Erect not branching
Old stem	Light brown	Grey	Light grey
Young stem	Yellowish green	Deep green	Deep green
Petiole	Bright red	Red	Green with violet tinge
Stipule	Blind small	Blind with violet base	Trifid prominent
Budleaf	Pale green	Pink	Light green
Age at flowering	12-14 months	6-8 months	Not flowered

Hybrid 63 R did not yield any results in back-crossing Extensive hybridisation work has also been conducted using these hybrid plants as male parents on a number of tapioca varieties as shown below

Female Parent	No of seeds
29 $\times$ 29 R	9
29 $\times$ 34 R	5
51 $\times$ 34 R	6
61 $\times$ 34 R	30
62 $\times$ 29 R	1
62 $\times$ 34 R	8
74 $\times$ 34 R	1
78 $\times$ 34 R	4
98 $\times$ 29 R	7
98 $\times$ 34 R	9
98 $\times$ 34 R	9

The behaviour of these seeds will be studied during the ensuing season One of the significant results of back-crossing has been the evolution of a few hybrid plants which are either non-flowering or self-sterile The agricultural importance of evolving sterile varieties by this method in a vegetatively propagated crop plant like tapioca, needs further investigation Although over 100 self-pollination trials were made, only one seed and that from 29 R has been secured In another set of trials these hybrids were used as female parents Tapioca variety No 38 on 32 R produced 6 seeds, No 29 on 36 R—2 seeds and No 38 on 36 R—2 seeds Detailed genetical study of these different categories of hybrid seeds will be undertaken during the ensuing cultivation

Interspecific hybridisation work on the lines detailed above appear to be very fruitful However promising the present results, the final selection of suitable strains has to await further yield trials The tubers formed in them are edible though more fibrous or woody Owing to the vigour in growth and the prolific tuber production in them, one can reasonably hope for promising results in this line of work

#### 4 Evolution of Polyploid forms

The diploid number of chromosomes in *M. utilis* is reported to be 36 Recent genetical work has established the fact that by inducing duplication of chromosome sets in certain cultivated plants, substantial increase in yield can be obtained Inducing duplication of chromosomes thus appeared another fruitful line of work for tapioca By repeated trials at Kayamkulam it was found that 5% solution of colchicine boiled

with agar agar was effective on young buds. The solution was applied nine times at intervals of three hours on buds of five varieties of tapioca. On variety No. 10 the solution was applied on ten of its cuttings. Four of these developed forms markedly different from one another and also the mother plant. They are recorded under numbers 2/10, 3/10, 8/10 and 10/10. Of these 8/10 is non-flowering while 3/10 and 10/10 are profusely flowering and 2/10 flowering only sparsely. The chromosome number of 2/10 has been ascertained as 72 ( $4n$ ) by Mr. A. Abraham. Its tubers are stouter but the rind is at least twice as thick and the starch grains much larger than in normal types, indicating that the chromosomes have been duplicated. Apparently divergent forms were obtained by this method on variety No. 3. Two distinct forms obtained from it were



FIG. 11 Variation in leaf forms of Polyploids

TABLE IV  
*Morphology of Polyploids*

Polyploids and normal dentatives	Growth habit	Old stem	Petiole	Leaflet	Shape of leaf	Colour of leaf	Flowering or not	Tuber characters	
								Skin	Rind
No 10 normal	Erect low branching	Grey	Red	Pink	Elliptic	Green	Flowering	White	Cream
Polyploid 2/10	Erect low branching	do	Red distal yellow towards the base	Green	Obovate	do	do	Brownish	Very thick reddish
Polyploid 3/10	Erect low branching	Light brown	Red	Deep pink (violet)	Elliptic	Deep green	do	Brown	White
Polyploid 8/10	Erect tall branching	do	do	Chlorophyllous	Elliptic	Green	Not flowering	White	White
No 20 normal	Erect low branching robust	Grey	Yellow	Green	Elliptic	Green	Flowering	White	White
No 20 polyploid	Erect tall branching vigorous	do	do	do	Obovate	Thick and deep green	No flower	Brownish	Cream
No 32 normal	Spreading	do	Yellow with red at top and base	do	Elliptic	Green	Flowering	White	Cream
No 32 polyploid	Erect low branching robust	do	do	Green	Obovate	Thick and deep green	do	White	White
No 36 normal	Erect low branching	Grey with brown tinge	Red	Pink	Lanceolate	Green	do	White	Cream
No 36 polyploid	Erect low branching	do	do	do	Obovate	Thick and green	do	do	do

found very promising in yield. These are included as 3P<sub>1</sub> and 3P<sub>2</sub> along with other high yielding varieties for further yield trials (*vide* Table II). Variety numbers 26, 32 and 36 were also subjected to this treatment and the forms derived from them are under observation. Fig 10 is a photograph of a normal No. 32 plant (N) with a polyploid (P) derived from it. Variation in leaf-form in polyploid types in the four varieties detailed above is shown in Fig. 11.

Owing to the minute size of chromosomes and the poor reaction of cells to most of the known fixatives the cytology of these polyploid forms has not yet been fully worked out. The marked variation of these forms in regard to external characters from their parent types is, however, indicative of chromosomal changes. The outstanding morphological characters of these forms in relation to their parent types shown below in Table IV would give an estimate of the extent of such variation. As in the case of hybrids continued selection work based on yield data should enable ultimate selection of outstanding forms for propagation.

### 5 Evolution of Triploids

The possibilities of evolving improved strains of cultivated plants by increasing their chromosome numbers are not fully known, but the improvements achieved in triploid varieties of apples and pears (Nilsson-Ehle, 1938) justify the hope that evolution of triploid varieties in tapioca may lead to the improvement of this crop plant. True tetraploid (4x) forms when crossed with diploid (2x) varieties give rise, among other methods, to what are known as triploids (3x). Although it has not been established that the colchicine induced forms obtained from tapioca are definitely tetraploids, the effect of crossing these plants with the varieties now under cultivation (diploid types) was worth investigation. A start has therefore

TABLE V

Morphology	Diploid	Triploid? 3/10 x 63	Tetraploid? 3/10
Growth habit	Tall branching	Non branching	Tall branching
Old stem	Grey with violet tinge	Light brown, nodes close set	Deep brown, node swellings prominent
Young stem	Green with red streaks	Dark green with red streaks	Yellowish green
Petiole	Red with violet tinge	Bright red drooping	Deep pink
Stipule	Prominent unbranched	1 fid with red base	Loloes with red base
Leaf	Broad middle	Broad middle	Broad distal
Budleaf	Green	Pale green with violet tinge	Pink
Age at flowering	4-5 months	Not flowered	6-8 months

been made with polyploid 3 10 as female parent and varieties 27, 28, 38 and 63 as male parents 10 plants were obtained and they are under observation in the Farm A typical leaf each of the tetraploid, the diploid and the triploid is shown in Fig 8 C The morphological features of these plants are given in Table V

#### 6 Evolution of pure varieties of Tapioca

The genetical behaviour of the different varieties of tapioca as also the large number of forms now cultivated in Travancore would make one

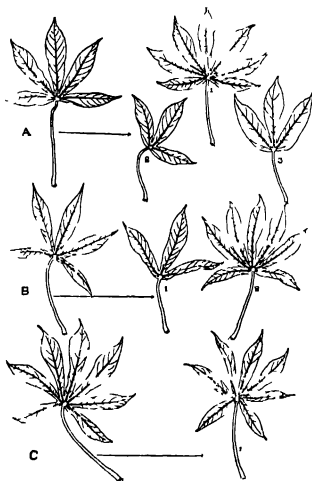


FIG 12 A Typical leaf of No 47 1, 2 3 types of leaves seen in the progeny of No 47 B. Typical leaf of No 49 1, 2 types of leaves seen in the progeny of No 49 C Typical leaf of No 74 1-typical leaf of plant of the progeny varying in external characters from No. 74.

suspect if all of them are true varieties. It is likely that chance hybridisation occurring in nature would become perpetuated as the plant is vegetatively propagated. One of the fundamental problems for solution therefore is to find out how many of the indigenous types of tapioca are true varieties breeding true to parental types and how many of them are only hybrids. Continued self pollination for successive generations of hybrid progeny would enable segregation of pure strains from hybrids. A start was therefore made this year on four varieties (Nos 38, 47, 49 and 74) by selfing them in order to study their genetical behaviour. 19 plants were obtained from No 38, 6 from No 47, 3 from Nos 49 and 2 from No 74. Segregation was clearly seen in the progeny of all of them. Extent of variation of leaf form characteristic of the different groups into which the progeny of these can be classified is shown in Fig 12. Outstanding morphological characters of the prominent types formed by this method are shown in Tables VI and VII.

TABLE VI

Morphology	Normal No 47	Type 1	Type 2	Type 3
Growth habit	Tall branching	Tall bran link	Profusely branching	Low branching
Old stem	Light brown	Brown	Dark brown	Brown with white streaks
Petiole	Red middle yellowish	Bright red middle yellowish	Light red middle yellowish	Light red middle yellowish
Leaf	5-7 lobed	3-7 lobed	3 lobed	7 lobed
Age at flowering	4-5 months	8-9 months	6-7 months	Not flowering
Male flower	Yellowish green	Sterile	Yellowish white	
Fruit wings	Prominent pink	Dark waxy stalk pink	Link waxy stalk pink	

Morphology	Normal No 49	Type 1	Type 2
Growth habit	Tall branching	Fruit not branched	Profusely branching
Old stem	Grey	Brown	Brown
Young stem	Green	Yellowish green	Yellowish green
Petiole	Upper red lower yellow	Red with middle yellow	Red with middle yellow
Stipule	3-fid red base	3-fid red base	2-fid red base
Leaf	5-7 lobed	7 lobed	2-3 lobed
Bud leaf	Pink	Green	Green
Age at flowering	4-5 months	Non flowering	6-7 months
Male flowers	Greenish white with reddish yellow disc		Open only partially greenish
Female flowers	Greenish yellow		Open partially by slit between perianth lobes
Fruits	Numerous		Few
Tuber	White thick	Reddish	Reddish

'Selfing' was repeated on one of the six plants of No 47 and 30 seeds have been obtained. One of the progeny of No 49 by selfing has given 12 seeds. In addition, varieties 17, 27, 56 and 98 have also been selected for selfing and 4, 143, 41 and 146 seeds respectively have been collected. These seeds will now be planted for further study.

#### VI CHEMICAL COMPOSITION OF THE TUBERS

According to an analysis made in the Bureau of Chemistry of the U S Department of Agriculture (Tracy, 1903), the chemical composition of dry tapioca tubers is as follows:

Constituents	per cent
Moisture	5.76
Ether extract	42
Crude fibre	5.08
Pentosan	2.63
Starch	64.28
Protein	2.98
Ash	1.96
Sugars, soluble cellulose, etc	16.89

The proportion of water in the fresh tubers varies with the nature of the soil and the time of harvesting. On an average about 66 per cent of the tuber is water. Hence the constituents of the fresh tubers will be:

Moisture	66.00
Ash	71
Protein	1.07
Crude fibre	1.83
Starch	30.24
Ether extract	15

As a food stuff the carbohydrates are largely in excess in tapioca with a nutritive ratio 1:28.5 instead of 1:7 which is accepted as the standard for a balanced diet. Fortunately Travancore does not lack fish and other nitrogenous food stuff rich in proteins. It should therefore be possible by a judicious combination of these with tapioca to have a balanced diet.

The results of analysis of tubers of 27 varieties of tapioca, conducted in the Division of Applied Chemistry (Public Analyst's Section) of the Central Research Institute of the Department of Research of the University, are shown below.

# The Tapioca Plant & Methods for Evolving Improved Strains 57

Sl No	Sample	Composition of raw tubers %			Composition of edible portion (no. of tubers)			Starch content of raw tuber %
		Round	Edible portion	Moisture	S	A	HCN	
1	1/10	14.4	85.6	68.7	81.7	2.10	0.94	21.9
2	2/10	22.5	77.5	75.4	85.7	2.80	1.73	16.4
3	3/10	13.0	87.0	67.4	86.2	1.6	.50	24.5
4	8/10	16.1	83.9	71.6	87.4	1.75	.64	16.6
5	10/10	14.1	85.9	68.7	81.7	9.15	.42	22.0
6	21	14.2	85.8	60	86.8	1.05	.31	9.4
7	22	18.3	81.7	0.1	82.9	1.8	.43	27.0
8	26	16.5	83.5	65.6	87.7	1.4	.39	24.0
9	28	16.0	84.0	74.8	81.1	2.10	.61	17.2
0	29	18.2	81.8	61.7	85.0	1	.18	26.6
1	30	21.1	78.9	53.8	82.3	.60	.32	30.2
2	32	14.1	85.9	78.7	90.1	1.7	.27	32.0
3	35	11.5	88.5	66.4	8	.7	.18	25.3
4	36	19.7	80.3	70.6	92.4		.42	19.4
5	38	16.1	83.9	58.6	81.7	1.8	.16	26.0
6	47	17.9	82.1	72.8	82.4	1.7	.88	18.4
7	49	18.6	81.4	67	84.8	1.7	.27	22.5
8	54	15.5	84.5	67.9	8.4	.6	.61	23.1
9	60	18.9	81.1	2.8	8.0	1.4	.79	5.8
10	67	17.1	82.9	6.8	78.1	1	.3	25.4
11	61	17.2	82.8	67.6	85.6	1.7	.21	27.6
12	62	15.0	85	61	85.3	.8	.2	20.7
13	67	17.8	82.2	58	85.1	1.35	.23	29.0
14	73	15.1	84.9	65.2	86.8	1.5	.28	25.6
15	74	15.7	84.3	68.5				—
16	75	15.1	84.9		81.4	1.40	.37	31.0
17	98	15.9	84.1	59.8	8.6	1.35	.45	27.2

Six out of the 27 samples have hydrocyanic acid content more than 50 mgm per 100 gms of dry tuber and it is significant that three of these are in polyploid forms. How far inducing polyploidy in this plant is associated with increase in HCN content is a matter for further investigation. It is also worth recording how one of the polyploid forms 2/10 with a tuber round about four times the normal thickness showed 173 mgm of HCN. This should mean that there is a probable correlation between roundness and thickness and HCN content in these tubers.

## VII SUMMARY

By the application of genetical methods detailed above a good number of new strains of tapioca and tapioca  $\times$  ceara hybrids have been produced. These strains are grown for yield trials with a view to selecting high yielding strains therefrom. The selected strains will soon be made available to the ryot for cultivation. Side by side with this line of investigation experiments are also undertaken to ascertain (1) best mode of planting seed canes (2) optimum spacing for planting (3) number of

plants per pit, (4) effective manures for the crop and (5) best period for harvesting. All these experiments are conducted on statistical design furnished by the Department of Statistics in the University. Owing to the fact that this line of work pertains to the agronomical aspects of this crop, it is proposed to embody its results in a separate paper. Meanwhile the above account of the applications of genetical methods for evolving better strains of tapioca, is presented as the first paper from the Tapioca Research Farm in order to stimulate further work on this important crop plant calculated to improve its cultivation in Travancore.

In conclusion the author wishes to record his grateful appreciation of the interest evinced by Rajyasevaprapina Dr K. L. Moudgill, Director of Research, in promoting this research work on tapioca. He also wishes specially to acknowledge the valuable contribution made by Mr A. Abraham, Economic Botanist now on deputation for advanced training in America, and the assistance rendered by the staff of the Research Farm, in the progress of this work.

Thanks are also due to Sachivottama Sir C. P. Ramawamy Aiyer, Dewan of Travancore and Vice Chancellor of the University, for the munificent endowment of Rs. 1,000 a month from which this work is being financed.

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 C H b 29 R bb n A<sub>b</sub> 8 n h



Fig. 9. Photograph of (1) Ceara rubber plant (A) and two hybrid plants obtained by Ceara  $\times$  Euphorbia cross.



Fig. 10. Plants in normal type plots.

# EXPLANATION OF TEXT-FIGURES AND PLATES

- Fig 1 The tapioca plant  $\times 1/20$  The soil level is shown by the line at base
- Fig 2 Young plant growing from a cutting  $\times 1/8$  Two buds have sprouted
- Fig 3 Flowers and fruit of tapioca  $\times 1$  A Inflorescence B 1 Female flower  
2 L S of same 3 Ovary showing basal disc 4 C S of ovary 5 L S of ovary  
showing obturator passing through micropyle of the ovule 6 Seed showing caruncle  
C 1 Male flower 2 Perianth removed showing the two whorls of flowers 3 One stamen  
enlarged 4 Disc
- Fig 4 Photograph showing three seedlings (Nat size)
- Fig 5 A Macroscopic view of the cut end of stem scl sclerenchyma B Microscopic view in T S  $\times 240$  Col—Collenchyma scl Sclerenchyma l v latex vessel xy xylem  
p pith C Latex vessel seen in tangential view l v latex vessel
- Fig 6 A T S of young root Cor Cortex End Endodermis Xy Xylem Ph Phloem,  
B Macroscopic view of the cut end of tuber C Microscopic view of T S sk Skin  
xy Xylem Pr py Protoxylem D Starch grains in cells of tuber E Different sizes of  
starch grains
- Fig 7 A Ceara rubber plant B Interspecific hybrid 63 Rubber Plant C Hybrid  
29  $\times$  R Age 18 months
- Fig 8 Variation in leaf forms in Interspecific hybrids
- Fig 9 Photograph of young Ceara rubber and interspecific hybrids A Ceara rubber  
plant B Hybrid seedling 74 R C Cutting from hybrid 34 R
- Fig 10 Photographs N Normal tapioca plant P Polyploid derived from it
- Fig 11 Variation in leaf forms of polyploids Leaf forms of polyploids are shown on  
the right and normal plants on the left Numbers represent variety numbers
- Fig 12 Variation in leaf forms in the self pollinated progeny of A Leaf of No 47  
1 2 3 represent types of leaves found in the different plants obtained by selfing it B Leaf  
of No 49 1 and 2 represent types of leaves in the progeny and C A leaf of No 74, with  
type of leaf in one of its progeny

## A NEW RUST ON *DALBERGIA PANICULATA* ROXB

BY T S RAMAKRISHNAN AND K RAMAKRISHNAN  
(Mycology Section Agricultural Research Institute Coimbatore)

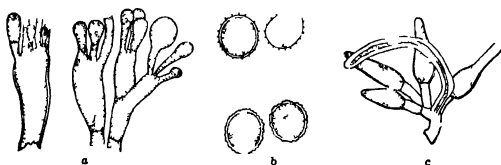
Received June 20 1947

(Communicated by Dr T S Sadasivan M Sc PhD, F A Sc)

In 1946 the writers collected a rust on *Dalbergia paniculata* Roxb from Walayar (Malabar District South India) An examination of the fresh material of the rust indicated that it was different from those already recorded on related hosts and it is described below as being new

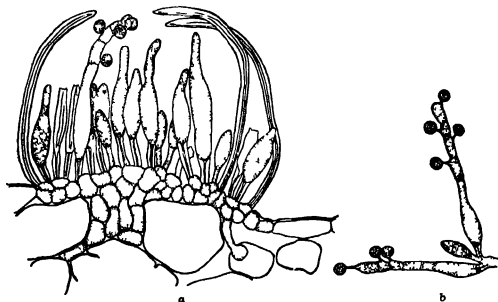
The uredia are hypophyllous or caulicolous, small and bright yellow The sori develop just below the epidermis which is burst through by the sporogenous cells The urediospores are globose or subglobose, stipitate, borne on short stalks which originate from the apices of cylindric branched or simple basal cells (Text Fig 1 a) Each basal cell gives rise to varying numbers of pedicellate urediospores Two kinds of urediospores can be distinguished One type has thin hyaline wall with prominent echinulations while the other has a thicker wall with less pronounced echinulations Both have yellow contents The proportion of the two kinds of spores varies in different sori and sometimes only the second type is present The spores measure  $15 \times 13 \mu$  ( $12-19 \times 9-14 \mu$ ) On the twigs also the sorus develops below the epidermis and bursts through this layer exposing the spores In the tissues of the branch the hyphae are seen to extend as far as the pith region (being present in the cortex and vascular tissue also) which suggests that the mycelium may remain in the tissue producing new crops of sori for a long time

Telia appear as buff to ochraceous buff, waxy, pulvinate, gregarious crusts amphigenously on the leaves and sometimes on the stem The teliospores are seen in old uredia also The exact place of origin of the telium is difficult to determine It seems to be intra epidermal forming a two to three-layered tissue from which basal cells project out above the level of the epidermis There is no evidence of the epidermis being pushed aside or burst through It is difficult to clearly differentiate the epidermal cells in the region of the sorus, but vague outlines of the epidermal cells containing the lower cells of the sorus can be made out in portions of the sorus Each basal cell gives rise to varying numbers of stipitate, oval to clavate, one-celled smooth and thin walled teliospores Incurved thick-walled paraphyses with narrow central lumen are found along the margin and



TEXT FIG 1 (a) Basal cells of uredium (b) Ured spores  $\times 500$  (c) Paraphysis and teliospores from common basal cell  $\times 300$

other portions of the telia. The origin of the paraphysis can be traced to the same basal cell which produces the teliospores (Text Fig 1 c). The teliospores measure  $15.30 \times 10-15 \mu$  (average  $19 \times 12$ ). They germinate immediately producing promycelia which are direct outgrowths of the apices of the spores. The promycelium is four celled and one round basidiospore is produced from each cell. The rust is a hemi form since it has only II and III stages.



TEXT FIG 2 (a) Section through a telium  $\times 530$  (b) Germinating teliospores  $\times 350$

*Maravalia achroa* (Syd.) Arth. and Cumm. has been recorded on *Dalbergia sissoo*. Type specimen of this material was obtained through the courtesy of Mr J. F. Dastur from the Indian Agricultural Research

Institute, New Delhi and examined. It was found that the telia of this rust were not paraphysate. The formation of the basal cells producing clusters of teliospores was not evident. Cylindrical basal cells developing groups of urediospores were not seen. In these characters it differs from the rust under study.

The formation of intra epidermal telia suggests an affinity to *Mainsia*. Though Jackson (1931) has recorded this genus on species of *Rubus* only, Thurmalachar (1947) has described *M. pterocarpi* on *Pterocarpus marsupium* from South India. In *Mainsia* however the urediospores develop singly, and not in clusters from free basal cells. Further the epidermal cells in the vicinity of the sori are said to be considerably hypertrophied in *Mainsia*. The rust on *D. paniculata* differs from *Mainsia* in these respects. The production of urediospores and teliospores in clusters from free basal cells indicates relationship to *Scopella*. But in the latter genus the sori are subepidermal and paraphyses have not been recorded in any of the species, whereas in the rust now described the telia are intra epidermal, and paraphysate. Thus it does not conform to any of the known genera of rusts and is therefore accommodated in a new genus *Scopellopsis* because of its resemblance to *Scopella* in the development of free basal cells bearing groups of spores in the uredia and telia. The rust on *D. paniculata* is described as *Scopellopsis dalbergiae*.

*Scopellopsis* gen. nov. Ramakrishnan, T. S. and K.

*Pycnia* and *aecia* not known, *uredia* subepidermal, erumpent, hypophyllous and cauliculous, *urediospores* subglobose, echinulate, pedicellate, produced in clusters from stout almost cylindric basal cells, *telia* amphigenous or cauliculous waxy, intra-epidermal in origin, projecting above the epidermis, *teliospores* stipitate formed in clusters from free basal cells, oval to clavate, one celled, germinating *in situ*, paraphysate, with incurved almost solid paraphyses.

Type Species *Scopellopsis dalbergiae* Ramak., T. S. and K. on *Dalbergia paniculata*.

*Pycnia* et *aecia* ignota, *uredia* subepidermia, erumpentia, hypophylla, caulicola, *urediosporidia* subglobosa, echinulata, pedicellata, producta in racemis ex cella crassa cylindrica simplici vel ramosa, *telia* amphigenia, vel caulicola, ceracea, plurimum intraepidermia, proijcientia super epidermem, *teliosporidia* stipitata, formata in racemis singulis cellis, ovalia vel clavata, unicellata germinantia *in situ*, paraphysata, paraphysibus incurvatis, ferme solidis.

Species typica *Scopellopsis dalbergiae* Ramak., T. S. and K., in vivis foliis et ramis *Dalbergiae paniculatae*.

*Scopellopsis dalbergiae* Ramakrishnan, T S and K, sp nov

*Pycnia* and *aecia* not known, *uredia* bright yellow, hypophyllous sometimes caulicolous, subepidermal, erumpent, minute, gregarious, pulvinate urediospores globose to subglobose, echinulate, with hyaline wall and yellowish contents,  $15 \times 13 \mu$ , stipitate, formed in clusters from simple or branched, stout, cylindric cells, telia amphigenous caulicolous, ochraceous buff, waxy, intraepidermal, spores projecting far above the epidermis, *teliospores* stipitate, formed in groups from free basal cells oval to clavate  $19 \times 12 \mu$  ( $14-30 \times 9-15 \mu$ ) germinating immediately *in situ*, paraphysate, with almost solid incurved paraphyses

On living leaves and stem of *Dalbergia paniculata* Roxb Walayar (Malabar), 31-12-46 T S Ramakrishnan and K Ramakrishnan

*Pycnia* et *aecia* ignota, *uredia* lucida flava, hypophylla et caulicola, subepidermia, erumpentia, minuta, gregaria, pulvinata, *urediosporidia* globosa vel subglobosa, echinulata, murus hyalini, contenta flavida,  $15 \times 13 \mu$ , stipitata, producta in racemis ex singulis cellis, crassa cylindrica, simplicia vel ramosa, *telia* amphigena, vel caulicola, silacei lutei colores, ceracea, intraepidermia, sporidia projicientia super epidermem, *teliosporidia* stipitata formata in racemis singulis cellis, ovalia vel clavata,  $19 \times 12 \mu$  ( $14-30 \times 9-15 \mu$ ) germinantia *in situ*, paraphysata, paraphyses incurvatis, ferme solidis

In vivis foliis et ramis *Dalbergia paniculata* Roxb Walayar (Malabar) 31-12-1946 T S Ramakrishnan et K Ramakrishnan

Type specimens of the rust have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and in Herb Crypt Ind Orient, New Delhi

#### ACKNOWLEDGMENT

The writers are indebted to Dr B B Mundkur of New Delhi and Dr G R Bisby of the Imperial Mycological Institute Kew, for their valuable criticisms and suggestions. They are also thankful to Mr J F. Dastur for kindly supplying the type specimen of *Maravalia achroa* (Syd) Arth and Cumm. Rev Fr Singarayay, Coimbatore, was kind enough to translate the diagnosis into Latin

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## REVISION OF A RUST ON *OLDENLANDIA* SPP.

By T S RAMAKRISHNAN AND K RAMAKRISHNAN  
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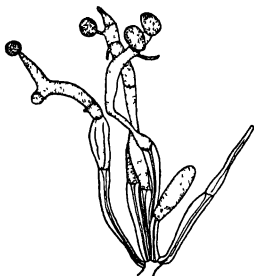
Received June 20, 1947

(Communicated by Dr T S Sadasivan M Sc, Ph D F A Sc)

In 1946 the writers collected rusts on *Oldenlandia stylosa* O Kze (*Hedyotis stylosa*, Br) and *Oldenlandia articularis* Gamble (*Hedyotis articularis* Br). The rusts on these two hosts agreed with the description of *Chrysocelis ascotela* (Syd) Thirumalachar. An examination of the fresh material of these specimens indicated that the taxonomy of this rust was in need of revision.

*Oldenlandia stylosa* O Kze is affected by a rust on the Nilgiris and Pulneys. One or more orange yellow spots which on drying turn black on the upper surface are formed on the leaf. On the upper surface of the spot several reddish brown to red pycnia are present. These are sometimes hypophyllous though more often they are epiphyllous. The pycnia are sub-globose, subepidermal deep-seated, and paraphysate with the paraphyses projecting out of the ostiole (Plate IX, Fig B). On the lower surface of the spots numerous crowded telia are seen. When fresh a wax-yellow to golden yellow colour is presented after the germination of the teliospores. Each telium is sunk in the tissue of the leaf. A peridium is lacking. The teliospores develop in oval broad cavities of the mesophyll (Plate IX, Fig C). They are one-celled clavate to cylindrical  $54 \times 13.6 \mu$  ( $37-70 \times 9-18 \mu$ ), pedicellate, thin-walled and filled with yellowish contents. The spores originate from a mass of hymenial cells, in the topmost layer of which the cells are laterally free and from each of which two, or more teliospores are developed. The spores of a cluster are of varying ages and all stages from initial formation to spores that have collapsed after germination can be seen in each group originating from a basal cell. Each spore has a pedicel which lengthens as the spore matures and may attain a length of  $40 \mu$  or more. When scrapings of the spores are examined under the microscope it is often seen that the pedicels are not complete but get partially broken. The pedicel is hyaline and has a central protoplasmic strand with clear gelatinising portion all round. Such pedicels have been described by Cummins (1940) for *Scopella bauhinicola*. Thus the spores are pedicellate and are produced in groups from laterally free basal cells.

The teliospores germinate *in situ* as soon as they are fully developed and at this time the stalk elongates to its maximum so that the promycelium is pushed out of the leaf tissue. The promycelium is the direct prolongation of the apex of the spore and does not come out through a germ pore. It is yellowish in colour, 4-celled and from each cell a big oval or round, thin-walled, light yellow basidiospore is produced on a sterigma. The protruding promycelia get reflexed on the epidermis and masses of these give a waxy appearance to the telia. The teliospores do not come out of the leaf at all but only the promycelia project out in a mass through a wide opening. The spore collapses after germination.



TEXT-FIG. 1 A cluster of teliospores of *Scopella ascotela* from basal cell  $\times 300$

This rust was first described by Sydow (1935) as *Blastospora ascotela*. Mains (1938) examined the specimen again and renamed it *Maravalia ascotela*. Thirumalachar (1942) studied it in greater detail and transferred it to the genus *Chrysocelis* as *C. ascotela*. Now our examination of fresh specimens collected at Ootacamund in October 1946 has shown the necessity for a further revision of the genus of the rust.

This rust is not *Blastospora* as is evident from the formation of the telia subepidermally. Thirumalachar includes the rust under *Chrysocelis* which has sessile teliospores, for he does not concede that the cell below the spore is a pedicel though he states that this elongates and reaches  $40\text{--}70\ \mu$  in length and "simulates a pedicel". In our opinion the cell below the spore is definitely a pedicel, reaching its maximum length when the spore germi-

nates That it is a pedicel is shown by its structure The central protoplasmic strand with hyaline gelatinising outer portion in this cell is indicative of its being a pedicel and not any other structure Such pedicels have been noticed in *Scopella* by Cummins (1940) Further it is hyaline and the spore is coloured With the collapse of the spore after germination the lower cell does not make any further growth but disintegrates in the same manner as remnants of pedicels often do Thirumalachar has stated that the spore is hyaline and he has not observed the difference in colour between the spore and its pedicel The colour of the spore is conspicuous in fresh specimens, but in old herbarium specimens, or two or three months after collection the colour is lost and this may be the reason why the colour has not been described by earlier authors Mains (1938) has also described the spore as hyaline Since the teliospores are prominently pedicellate the rust cannot be *Chrysocelis* In *Maravalia* the teliospores are produced singly from the cells of a compact hymenium (Mains, 1939) In the rust under study examination of microtome sections and dissected telia showed that the teliospores are formed in groups of varying numbers, each group developing from a basal cell which is laterally free (Plate IX, Fig A) That the cells are laterally free can be clearly seen in the photomicrograph of a cluster where a small spore is developing from a side of the basal cell which will be possible only if the basal cells are laterally free Each cluster contains spores in different stages of development For these reasons this rust cannot be included in the genus *Maravalia* Judging from the characters of the telia and the teliospores it must be transferred to the genus *Scopella* Mains (1939) who founded the genus has stated that in *Scopella* the pycnia are subcuticular and hemispherical while in this rust they are subepidermal and subglobose This difference need not be a serious objection to include this rust under *Scopella* Instances are known in other genera [e.g., *Ravenelia* (Arthur, 1934)] where both kinds of pycnia have been observed in the same genus

The distinction between *Scopella* and *Maravalia* rests mainly on, (1) the compactness or lateral freedom of the basal cells and (2) the formation of one or more teliospores from each basal cell Cummins (1940) states that the basal cells are subject to variation and that there is no rule by which one can definitely decide when basal cells cease to be basal cells and become part of a compact hymenium Considering the variation that may be expected in the hymenial layers and taking into account that we are dealing with living organisms in which machine-made uniformity cannot be expected, it is quite possible that *Maravalia* and *Scopella* are merged into one and the same genus, at a later time, For the present this merger is not attempted

and as the rust does not fit in with Mains' emended diagnosis of *Maravalia* but agrees more with *Scopella* it is thought fit to place this rust in the latter genus and revise the name as *Scopella ascotela*

A similar rust was found infecting the leaves of *Oldenlandia articulatis* in the neighbourhood of Ootacamund and the Agricultural Research Station, Nanjanad, Nilgiris. Only telia were present and these formed deep-pink, waxy, raised patches on the lower surface of the leaves. Corresponding brown areas became visible on the upper surface at a later stage. Telia are subepidermal and sunk in the tissue. Teliospores are one-celled, deep orange coloured with thin hyaline walls,  $45.5 \times 12.8 \mu$ , formed in clusters from free basal cells, pedicellate, pedicels hyaline  $36-65 \times 10-14 \mu$ , with a central protoplasmic strand and hyaline gelatinising outer portions. Teliospores germinate *in situ*, producing apical promycelia which project beyond the surface of the leaf.

This rust closely resembles the one on *O. stylosa*, the difference being the absence of pycnia and the difference in the colour of the telia and the teliospores. These differences do not warrant the creation of a new species though the host is different. As the structure of the telia and the teliospores and the spore size do not exhibit any significant difference, this rust is also identified as *S. ascotela*.

The diagnosis of the genus *Scopella* is emended as follows—*Pycnia* amphigenous, subcuticular and subepidermal, hemispherical or globose, *uredia* subepidermal, powdery, *urediospores* brown, pedicellate, several arising together from a cylindrical basal cell, basal cells free amongst themselves, *telia* subepidermal, *teliospores* unicellular, pedicellate many arising from a single basal cell, basal cells free amongst themselves, spore wall thin, hyaline, without germ pore, teliospores often coloured, germinating *in situ*, at once by apical prolongation of the teliospore.

*Scopella ascotela* (Syd.) Comb. nov. Ramakrishnan and Ramakrishnan

Synonyms *Blastospora ascotela* Syd.

*Maravalia ascotela* (Syd.) Mains

*Chrysocelis ascotela* (Syd.) Thirumalachar

*Pycnia* amphigenous mostly epiphyllous, grouped in the rust spot, subepidermal, subglobose, sunk in the tissue  $155-207 \times 110-125 \mu$ , *uredia* and *aecia* not known, *telia* hypophyllous, subepidermal, clustered in the region of the spot which is thickened, waxy-yellow to golden yellow or pink, *teliospores* one celled with yellowish or orange coloured contents, clavate to cylindric, pedicellate pedicels hyaline up to  $65 \mu$  long, produced in groups

on laterally free basal cells, the spores in a group being in different stages of development,  $54 \times 13.6 \mu$  ( $37-70 \times 9-18 \mu$ ) germinating *in situ* by the prolongation of the spore apex into ephemeral yellowish 4-celled promycelium, basidiospores round or oval, light yellow.

On living leaves of *Oldenlandia stylosa* O. Kze. and *Oldenlandia articulatis* Gamble. Ootacamund, Nilgiris, 22-9-1946 (T. S. Ramakrishnan).

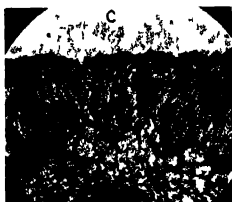
We gratefully acknowledge the help received from Dr B. B. Mundkur, New Delhi, and from Mr. K. M. Thomas, Government Mycologist, Coimbatore, in critically reading through the manuscript and offering suggestions.

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#### EXPLANATION OF PLATE

- A. One cluster of teliospores on a basal cell, ( $\times 500$ ).
- B. Pycnium, ( $\times 500$ ).
- C. Telia, ( $\times 100$ )





# AERATION AFFECTING GROWTH AND SPORULATION OF SOME SOIL *FUSARIA* IN LIQUID CULTURES

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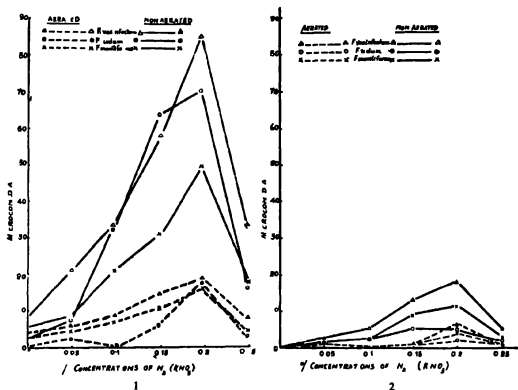
## INTRODUCTION

THE effect of aeration on the growth and sporulation of the genus *Fusarium* has not been studied in detail, but it is commonly recognised that aeration removes the stale gases of metabolism and ensures a pure supply of oxygen thereby accelerating the growth of a fungus both in the soil and in a pure culture medium

Almost the first convincing evidence on the importance of aeration in the spread of soil-borne fungal infections of plants was presented by Garrett (1937). Indeed, Garrett (1936) classified *Fusarium vasinfectum* and other *Fusaria* under "diseases favoured by light soils". Experimental proof was presented by him (1936) in a decrease in growth rate of the fungus *Ophiobolus graminis* when sand was mixed with an inert substance like pure china clay, the decrease being conditioned by the decrease in soil aeration. However, evidence of a similar kind on the pure culture side had been lacking and, therefore, a study was made with *Fusarium vasinfectum*, *F. moniliforme*, and *F. udum* (isolates from cotton, paddy and pigeon pea root-rot) by growing these fungi in aseptically aerated liquid culture solutions containing various organic and inorganic nutrients. The results have generally confirmed previous observations on the soil conditions and the occurrence of *Fusarium* root infections.

## MATERIAL AND METHODS

The standard synthetic medium of Horne and Mitter's (glucose = 2 gm, potassium nitrate = 2 gm, potassium phosphate (tribasic) = 1.25 gm, magnesium sulphate = 0.75 gm, starch = 10 gm, distilled water = 1000 ml) was used throughout without the agar. The standard medium contained 0.028 gm of nitrogen in the form of potassium nitrate per litre and not asparagin as the source of nitrogen. All the strains of fungi used were pure culture isolates and were type cultures received from authentic sources.



TEXT FIGS 1 & 2—Fig 1 Shows micro-conidial production at varying nitrogen levels in aerated and non aerated series in the three *Fusaria*. Fig 2 Shows macro-conidial production at varying nitrogen levels in aerated and non aerated series in the three *Fusaria*.

Aeration was effected by connecting in a series 250 ml Erlenmeyer flasks, containing the sterilised liquid cultures previously inoculated with the fungus, to an air pump. The rate of flow of the air was adjusted at 660 ml per minute and the incoming current of air was made aseptic by passing through disinfectants contained in Woulff's flasks. The experiments were carried out at laboratory temperature, which fluctuated between 25° C–30° C. Aeration was started twenty four hours after inoculation, 0.1 cc of inoculum from liquid cultures being added to each flask. Spore numbers were determined quantitatively, the counts being taken under oil immersion by shifting the field to one division of the stage vernier in any of the four directions in a coverglass area of 7/8 sq."

For quantitatively determining the growth of the fungi, fungal mats were removed at two different periods of growth viz, 10 days and 21 days after inoculation, filtered and subsequently dried in the oven at 70°–80° C, to constant weights. They were then incinerated to determine ash weights.

## EXPERIMENTAL

## I Effect of aeration on sporulation at various nitrogen levels

A combined experiment was set up to find out what effect aeration and variation in total nitrogen had on sporulation. Flasks containing 50 ml of Horne and Mitter's medium with varying concentrations of nitrogen were aerated, aeration being started 24 hours after inoculation and conducted intermittently at the rate of two running hours per day. Another series of non-aerated control flasks was maintained and cultures were examined one week after inoculation and the results are graphically presented in Text-Figs 1 and 2.

These figures show that sporulation is optimum at 0.2% potassium nitrate concentration in both the aerated and non aerated series. However, aeration is detrimental to optimum spore production (both micro- and macroconidia) in any concentration of total available nitrogen.

Microconidia are prolific whereas, macroconidia are very few in comparison and among the three fungi *F. vasinfectum* shows higher micro- and macro-conidial formation than the other two species.

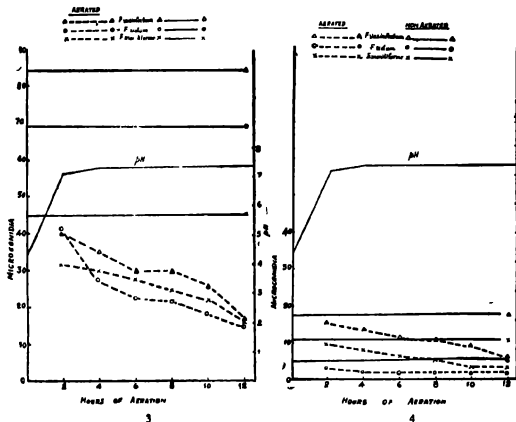
## II Effect of aeration on sporulation and pH of culture medium

As root infecting fungi are in general strong aerobes, it was doubtful whether the depressing effect on sporulation could be attributed to the direct effect of aeration. It seemed more probable that continuous aeration possibly had something to do with altering the pH of the medium to an unfavourable level resulting in reduced sporulation. Therefore, examination of cultures for pH values before and after specified hours of aeration was undertaken and an experiment started to find out whether aeration altered the pH in any way.

For each species of *Fusarium* six flasks containing liquid cultures were used and connected to the air pump, thus having three series, of six flasks for each fungus, with similar numbers for the non-aerated control. Initial pH values were taken prior to aeration for both the series. All the 3 series were aerated for two hours, one flask from each series being detached and pH values taken for the three fungi whilst determining at the same time pH values for the control series. It must be mentioned here that aseptic aeration for 2, 4, 6, 8, 10 and 12 hours of uninoculated media does not in any way alter the pH as compared with the non-aerated series. However, with fungus inoculum growing, the aerated series did show rise in pH values but this rise from the acidic side, i.e., pH 4.4 to pH 7.4 was similar both in aerated and non-aerated series. Along with final pH readings which were

taken on the seventh day after detachment for aerated cultures, spore counts were made. Control series were also examined for spore numbers. This evidence tended to show that duration of aeration had no direct effect on the pH of the media but still produced profound changes in the number of micro- and macro-conidia produced, which effect is attributable, therefore, directly to aeration.

The results are graphically presented in Text-Figs. 3 and 4



Text-Figs 3-4—Fig 3 Shows micro-conidial production by the three *Fusaria* at different hours of aeration. Changes in pH are also presented. Fig 4 Shows macro-conidial production by the three *Fusaria* at different hours of aeration. Changes in pH are also presented

Text-Figs. 3 and 4 show that

1. With increase in number of hours of aeration, spore production is on the decline.
2. Percentage fall in sporulation on two hours aeration is highest in *F. vasinfectum*, followed by *F. udum* and lowest in *F. montiliforme*.

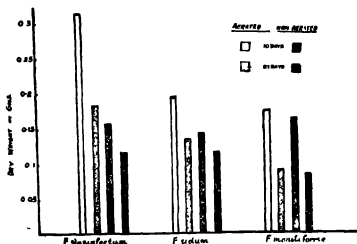
3. Macro-conidia as usual are fewer in number than micro-conidia which appears as a uniform characteristic in all the three species.

4. Maximum aeration brings about a marked fall in all the three cases.

5. Duration of aeration has no effect on the pH of the culture media.

### III. Effect of aeration on weight of fungus.

The fall in micro-conidial and macro-conidial numbers noticed in Experiment I under aerated conditions in liquid media containing varying amounts of nitrogen necessitated the study of the behaviour of the mycelia of the three species of *Fusarium* from the quantitative point of view. It has been shown recently in this laboratory by one of the authors (Yogeswari, unpublished) that variation in the dry and ash weights of *Fusaria* growing in liquid cultures with different nutritive substrates, can be very accurately determined quantitatively, with suitable replications within treatments. Thus, it was noticed that aeration of the liquid cultures considerably increased both dry and ash weights of the mycelial mats in all the three species of *Fusarium* under investigation. The details of the results of this experiment are presented diagrammatically in Text-Figs. 5 and 6.



TEXT-FIG. 5. Shows dry weights of the three *Fusaria* after 10 days and 21 days growth in aerated and non-aerated cultures.

Text-Fig. 5 shows that

1. Dry weights of the three *Fusarium* spp. in aerated and non-aerated cultures taken after ten days are higher than those determined after twenty-one days.

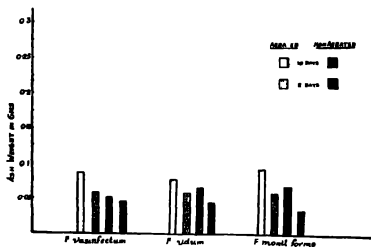
2 (a) *F. vasinfectum* in both ten-days old and twenty-one days old aerated cultures shows better growth over the other two fungi

(b) *F. moniliforme* shows lowest weight

(c) In non aerated ten day old cultures *F. moniliforme* shows a slight increase in dry weight over *F. vasinfectum* and *F. udum*

(d) In non-aerated twenty one day old cultures fall in *F. moniliforme* is very marked being lower than *F. vasinfectum* and *F. udum* dry weights

3 Difference in dry weights between ten and twenty-one day old aerated cultures of the three *Fusaria* is very pronounced in *F. vasinfectum* while in non aerated cultures, difference in dry weights is most marked in *F. moniliforme*



TEXT FIG. 6 Shows ash weight of the three *Fusaria* after 10 days and 21 days growth in aerated and non aerated cultures

Text-Fig. 6 shows that

1 Ash weights of the three *Fusaria* in aerated and non-aerated cultures taken after ten days are higher than those taken after twenty-one days but unlike in Text Fig. 5, all the strains show almost level weights in ten-day old cultures. The fall in weight after twenty-one days is highest in *F. moniliforme* in both the series

2 Fall in the ash weights after twenty-one days growth is not so great as compared with the dry weights

#### DISCUSSION

Aeration of liquid culture media under the present experimental conditions retarded the spore producing ability of *F. vasinfectum*, *F. moniliforme*

and *F. udum*. On the other hand, very distinct increase in dry and ash weights of the three species was observed under similar conditions of aeration over their non-aerated controls. It was felt at the time when these results were obtained that aeration of liquid cultures possibly introduced some changes in the reaction of the medium, which in turn resulted in the inhibition of the spore producing ability followed by an increase in mycelial mat formation. But further experiments showed that the pH of both aerated and non-aerated series ran parallel with each other, although shifting the reaction from the acidic to alkaline in both cases. It was further thought that changes in the availability of total nitrogen possibly governed spore production. But even here it was discovered that increase or decrease in total nitrogen did, doubtless, bring about changes in the quantity of spore produced, but the general spore-producing ability of the fungi was still very low in the aerated as compared with the non-aerated series. These experiments have brought to light the functions of aeration in increasing vegetative growth of *Fusaria* as well as in delimiting their ability to sporulate. Valuable comparisons can be made with already established facts that light sandy soils promote ramification of mycelia induced by conditions of abundant soil aeration and it is significant to note that the more rapid disappearance of various soil fungi in light soils is possibly due to the poor spore-forming tendency of the fungi concerned. Further work on soil isolations from cotton-growing tracts with light soils (which is in progress) may confirm the finding which has been conducted under pure culture.

That saprophytic fungi are most active in the decomposition of plant residues under conditions of abundant soil aeration is an established fact (Waksman, 1931, Garrett, 1938, 1939). The purport of this paper is mainly to emphasize that these findings on the behaviour of soil *Fusaria* in pure culture, both aerated and non-aerated is in keeping with established facts that disappearance of various soil fungi is most rapid in loose soils possibly due to their inability to sporulate normally. The vegetative growth under such aerated conditions although better than non-aerated does not contribute towards the longevity of the fungus, since the inevitable micro-biological antagonism shortens the vegetative phase of fungal activity more easily than when confronted with prolific spore development, the latter being less vulnerable to micro-biological attack.

#### SUMMARY

1. Effect of aseptic aeration on growth and sporulation of the three soil fungi, viz., *F. vasinfectum*, *F. moniliforme*, and *F. udum* was studied in detail.

2 Sporulation of *F. vasinfectum*, *F. moniliforme* and *F. udum* was optimum at 0.2% nitrate nitrogen in standard Horne and Mitter's liquid medium

3 Aeration stimulated mycelial growth (on both dry and ash weight basis) but inhibited sporulation (quantitatively determined)

4 Aeration had no direct effect on the pH of the culture medium

5 Sporulation decreased with increasing hours of aeration

#### ACKNOWLEDGMENT

We are greatly indebted to Dr T S Sadasivan for his helpful criticism and guidance in the course of this investigation

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NOV 1947

**FUSARIUM SP PARASITIC ON EPIPYROPS, A  
LEPIDOPTEROUS PARASITE OF THE SUGARCANE  
PYRILLA**

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(Communicated by Prof L. Narayana Rao I A S C)

INTRODUCTION

In October 1939 the late Dr John Muliya brought to the writer's notice pupæ of *Epipyrops* sp attacked by a fungus. In its larval stage *Epipyrops* is a parasite on *Pyrilla* the well known pest of sugarcane. A study was undertaken to identify the fungus and to establish its pathogenicity on *Epipyrops*.

DESCRIPTION OF THE MATERIAL

The pupæ are rectangular in shape 22-25 mm long and 8-9 mm broad and were attached to the ventral surface of the sugarcane leaf the longer axes of the pupæ being along the length of the leaf. The pupæ were covered over by salmon coloured mycelium. When a portion of the mycelium was examined under the microscope innumerable conidia of *Fusarium* sp were observed.

IDENTIFICATION TESTS

The morphology of the fungus was studied in detail. The characters of the conidia produced on the mycelium parasitising *Epipyrops* were compared with those produced by single spore cultures isolated from the former. A remarkable variability was at once perceived in the spores produced in culture. One of the single spore isolates differed from the fungus obtained directly from *Epipyrops* in the total absence of macroconidia, while both the micro and macro conidia were present in other isolates.

Three isolates were used in the study of the morphology of the fungus: (i) the fungus from *Epipyrops*, (ii) a single conidial culture from (i) but producing only microconidia, (iii) a single conidial culture from growths isolated from artificially infected *Epipyrops* pupæ [the healthy *Epipyrops* pupæ were artificially infected in the laboratory with spore suspensions of (i) above].

The two isolates, (ii) and (iii) were grown on the following seven media

- 1 Potato cylinders
- 2 2% potato Dextrose agar
- 3 5% potato Dextrose agar (Wollenweber, *et al*, 1925)
- 4 Oatmeal agar
- 5 Steamed rice (Wollenweber *et al* 1925)
- 6 Brown's standard agar (Brown, 1925)
- 7 Brown's starch agar (Brown, 1925)

The potato cylinders were prepared in the usual manner and were sterilized at 10 lbs pressure for 45 minutes

The oatmeal agar was prepared by taking 100 grams of Quaker Oats in water, using a sufficient quantity of water to bring the oats into solution when warmed. The warm solution was strained through cheese cloth, taking care that no crushing or pressure was applied to help the slow streaming through of the solution. The solution was made up to 1000 cc, tubed, and sterilized at 10 lbs pressure for 45 minutes

Triplicate tubes of the cultures grown in the seven media were maintained at two temperatures, 18-20°C and 35°C, to study the range of variation exhibited by the fungus, and to identify it on the basis of the characters so observed taken in conjunction with the morphology of the fungus from the naturally occurring parasite on *Epiphyrops*. For convenience, the two cultures will be referred to in the following text as culture 1, and culture 2, while the *Fusarium* sp. from *Epiphyrops* will connote the fungus obtained directly from the parasitised material

Observations were made on the 10th and 21st day on the amount and colour of aerial mycelium and substrate, production of sclerotia stroma and pionnotes. The data are recorded in Tables I and II

The spore measurements and data on the percentage occurrence of the different septate spores were obtained on the 22nd day in culture 1, and on the 23rd and 24th days in culture 2. The spore measurements, etc., were made only on the cultures maintained at 18°C. The data are presented in Tables III, IV, V, VI. The spore measurements data for *Fusarium* sp. from *Epiphyrops* are shown in Table VII

The data presented in the tables may be summarised as follows. In both the cultures, the aerial mycelium is colourless or white.

TABLE I

Showing the amount of Aerial Mycelium, colour of Aerial Mycelium and of Substrate, Conidial Production Sclerotia, Stroma, Pannotes on the 10th and 21st days of growth at two Temperatures in different media

Medium	Culture	Temperature series °C	Aerial Mycelium		Surface of substratum		Sclerotia Stroma	Pannote
			10th day	21st day	10th day	21st day		
Potato cylinder	I	18	White	White abundant 0.3 septate spores abundant	Unilateral	Olive grey	Absent	Absent
		35	White	White abundant 0.2 septate spores abundant	Dark olive grey	Deep olive green grey	Absent	Absent
	II	18	White	White abundant 0.5 septate abundant	Light green conical	Buff pale deep lilac blue green scattered	Absent	Absent
		35	White	White abundant	Aquatic blue	Apricot yellow scattered on the surface	Absent	Absent
2% Potato Dextrose	I	18	White	White and deep dull bluish violet moderate abundant conidia 0.1 septate abundant	Lilac violet	Deep violet brown conidia scattered 0.1 septate	Absent	Thin
		35	Lacking	Lacking	Orange pink dark nigrosin violet	Cinnamon buff dark nigrosin violet conidia 0.1 septate scattered	Absent	Thin
	II	18	White	White scanty conidia 0.6 septate	Salmon buff	Salmon buff conidia 0.6 septate	Absent	Thin
		35	Lacking	White scanty conidia few, 0.3 septate mostly	Flashed ochre	Flesh chie, abundant conidia 0.5 septate	Absent	Thin

TABLE I (Contd)

Medium	Culture	Temperature series 0°C	Aerial Mycelium		Surface of substratum		Sclerota Stroma	Pinnater
			10th day	21st day	10th day	21st day		
5% Potato Dextrose	I	18	White	White moderately abundant as in 27	Unchanged	Deep slaty brown	Absent	Thin
		35	Lacking	Lacking	Dark nigrosin violet pink like Cinnamon	Dark nigrosin buff pink conidia scattered 0.1 septate	Absent	Thin
	II	18	White	White	Salmon buff	Salmon buff	Absent	Thin
		35	Lacking	White scanty conidia as in 27	Flesh colour	Flesh ochre	Absent	Thin
Oatmeal Agar	I	18	White	Lacking	Unchanged	Hyssop violet, argyle purple Conidia 0.1 septate	Absent	Thin
		35	White trace of faint red	Lacking	Unchanged	Unchanged, Conidia abundantly 0.1 septate	Absent	Thin
	II	18	White	White moderately abundant conidia abundant 0.7 septate	Salmon colour	Apricot buff Conidia scattered	Absent	Thin
		35	Lacking	White abundant conidia abundant mostly 0.3 septate	Salmon buff	Salmon buff, Conidia scattered	Absent	Thin
	I	18	White	White abundant conidia 0.2 septate	Cameo pink	Thalite pink	Absent	Absent
		35	White	White abundant 0.2 septate abundant	Coral pink and Apricot orange	Coral pink ochraceous orange, wax yellow	Absent	Absent

TABLE I—(Contd)

Medium	Culture	Temperature series 0°C	Aerial Mycelium		Surface of substratum		Sclerotia stroma	Finneter
			10th day	21st day	10th day	21st day		
Steamed Rice	II	18	White	White abundant, conidia abundant 0.5 celled, mostly 0.5 septate	Salmon colour deep dark olive buff	Conidia thinly scattered	Absent	Absent
		35	White	White scanty (Bacterial contamination) conidia mostly 2.5 septate	Fresh ochre	Apricot buff cream buff orange at the bottom, then red at the top and common buff	Absent	Absent
Brown's Standard Agar	I	18	White	White scanty conidia abundant, septate mostly 2.5 septate	Unchanged	Unchanged conidia scattered 0.5 septate	Absent	Absent
		35	Lacking	Lacking	White	White conidia abundant septate 0.5 septate	Absent	Absent
	II	18	White	White scanty conidia 0.5 septate abundant	Lacking in colour	Lacking in colour conidia thinly scattered	Absent	Absent
		35	Lacking	Lacking	Apricot buff	Apricot buff abundant conidia mostly 0.5 septate	Absent	Absent
Brown's Starch Agar	I	18	Lacking	Lacking	Unchanged	Fumy blue abundant conidia 1.2 septate	Absent	Thick
		35	Lacking	Lacking	Prussian blue	Dull purplish black abundant conidia 1.2 septate	Absent	Absent
	II	18	White	White scanty conidia 0.5	Pale salmon	Salmon, conidia, thinly scattered on the surface	Absent	Absent
		35	White	White scanty conidia mostly 0.3 septate	Apricot orange	Apricot buff, conidia abundant	Absent	Absent

TABLE II

*Showing the form of Conidia and Chlamydospores observed on the 10th and 21st days of growth at two different Temperatures on Seven Different Medium*

(5% Pot dextrose not shown as the data were similar to those in 2% potato dextrose)

Medium	Culture	Temperature °C	Conidia in aerial mycelium	Conidia on the stomatal layer	Chlamy- dospores
Potato cylinder	I	18°C	Single continuous and 1 9 septate 4 septate spores comparatively abundant ovoid to spindle shaped cylindrical slightly curved, sometimes slightly v. culate apex rounded		Absent
	I	38°C	Single continuous 1 3 septate spores ovoid spindle shaped cylindrical or slightly curved hyaline abundant		do
	II	18°C	Single continuous 1 7 septate mostly 3 and 5 septate ovoid to cylindrical microconidia macroconidia straight or rarely curved bluntly pointed ends without a foot cell	Spores scattered over the surface surface slightly slimy	do
	II	38°C	Single continuous predominantly septate 1 5 septate mostly 3 septate microconidia ovoid to cylindrical or curved microconidia sickle shaped spindle shaped cylindrical straight with blunt ends slightly foot celled base curved spores with a fine curved point	Spores abundant on the slimy surface	do
Potato Dextrose	I	18°C	Single continuous (1 2 septate spores also seen) ovoid to spindle shaped hyaline abundant	Thinly scattered on agar surface predominantly continuous ovoid to spindle shaped occasionally 1 3 septate thin straight or slightly curved and rounded apex and bluntly pointed or slightly foot celled base separations indistinct hyaline	do
	I	38°C	do	do	do
	II	18°C	Single continuous and 1 septate microconidia ovoid to spindle shaped or straight cylindrical macroconidia curved straight or slightly curved with bluntly pointed ends or finely pointed ends	Spores thinly scattered on the agar surface, rarely with slightly foot celled base	do

TABLE II—(Contd)

Medium	Culture Temperature °C	Conidia in aerial mycelium	Conidia on the stomatal layer	Chlamydo- spores
Potato Dextrose	II 35°C	Predominantly septate straight with blunt ends curved slightly foot celled base mostly 0-3 septate rarely 4-15 septate	Abundantly scattered over the surface ovoid microconidia typically curved in 1 straight 0-5 septate frequently curved ends blunt or bluntly pointed with or without a foot cell base a distinctly septate	Absent
	I 18°C	Single continuous rarely septate ovoid cylindrical spindle shaped rounded apex and base, not abundant	Thinly scattered on Agar surface mostly conidium is rarely 1 septate curved cylindrical spindle shaped rounded apex also blunt without any foot cell conidia comparatively not abundant	do
	I 35°C	Single continuous rarely 1-2 septate spores ovoid cylindrical spindle shaped hyaline abundant	Thinly scattered on agar surface spindle shaped occasionally 1 septate straight slightly curved apex blunt or rounded septations indistinct hyaline	do
Oatmeal Agar	II 18°C	Conidia 0-7 septate mostly septate spores 3-5 septate spindle shaped elongated slightly pointed ends curved, slightly foot celled microconidia ovoid cylindrical rounded apex	Thinly scattered on the surface	do
	II 35°C	Conidia 0-5 septate mostly 0-7 septate ovoid to spindle shaped stout thick and straight or narrow and slightly curved the ends blunt or finely pointed hyaline occasionally vacuolate	Thinly scattered on the surface	do
Steamed Rice	I 18°C	Single continuous mostly occasionally 1 septate, rarely 2 septate ovoid to spindle shaped curved straight cylindrical apex rounded blunt abundant	Spores thinly scattered on the surface	do
	I 35°C	do	do	do
	II 18°C	Conidia 0-5 celled mostly 1-5 celled spindle shaped straight or slightly curved ends blunt or bluntly pointed, hyaline occasionally vacuolate	Spores thinly scattered on the surface	do
	II 35°C	Conidia very irregularly shaped mostly 0-3 septate rarely 4-5 septate, spherical to ovoid and spindle shaped with blunt ends	Abundantly scattered on the surface	do

TABLE II—(Contd)

Medium	Culture	Temperature series °C	Conidia aerial mycelium	Conidia on the stomatal layer	Chlamydo spores
Brown's Starch agar	II	18°C	Conidia very thin walled 0.5 septate ovoid to spindle shaped blunt or bluntly pointed ends	Conidia thinly scattered on the surface	Absent
		35°C	Conidia 0.5 septate mostly 0.3 ovoid to cylindrical spindle shaped blunt or bluntly pointed	Conidia abundantly scattered over the surface	do
	I	18°C	Single continuous very rarely 1 septate ovoid to spindle shaped occasionally curved apex blunt abundant with or without foot celled base		do
		35°C	Single or false heads ovoid to cylindrical 1.2 septate straight and slightly curved apex rounded without foot cells abundant hyaline		do
	I	18°C	Single predominantly septate 0.3 septate mostly straight ovoid to spindle shaped occasionally slightly curved apex rounded or pointed very distinct septate abundant	Single continuous 1 septate occasionally 2 septate spindle shaped ovoid rarely curved apex rounded or pointed septate distinct abundant	do
		30°C		Septate spores comparatively abundant 1.3 septate ovoid cylindrical furrowed curved to sickle shaped apex blunt or bluntly pointed base slightly foot celled	do
Brown's Standard Agar	II	18°C	Conidia thin walled 0.5 septate mostly ovoid to spindle shaped septate spores curved with blunt or bluntly pointed ends	Spores thinly scattered on the surface	do
		35°C		Single continuous or septate 0.3 septate ovoid to cylindrical or spindle shaped blunt to pointed ends curved to sickle-shaped without foot cell—conidia typically like those of I in 18°C and 35°C	do

TABLE III

*Showing the Percentage Occurrence of the different septate spores in the Seven Media of Culture I at 18° C (after 23 days)*

Medium	0 septate	1 septate	2 septate	3 septate
Potato cylinder	96	4	..	..
2% P.D. Agar	98	2	trace	trace
5% P.D. Agar	..	..	..	..
Oatmeal	96	2	..	..
Steamed rice, medium	98	2	trace	..
Brown's starch, medium	96	2	..	..
Brown's standard medium	98	2	..	..

TABLE IV

*Showing the mean measurements in  $\mu$  of length, the range in length of the Conidia of Culture I at 18° C. after 23 days*

Medium	0 septate		1 septate		2 septate	3 septate
	Mean	Range	Mean	Range		
Potato cylinder	8.94	4-16	13.52	10-20		
P.D.A. 2% ..	7.28	4-12	15.80	8-24	18.4	32
Oatmeal ..	8.68	4-10	14.6	10-24	22.8	20
B.A. ..	8.4	4-16	18.0	12-24		
Blot. A. ..	6.4	2-12	13.52	10-20		
Rice ..	8.84	4-16	15.72	10-24	19	24
	8.1	2-16	15.4	8-24	20.1	25.3



TABLE VI

*Mean measurement and percentage occurrence of Septate Spores in Culture II*

Septation	0	1	2	3	4	5	6	7
Percentage	3%	6%	7%	52%	8%	23%	1%	Trace
Mean length	9.59	14.25	18.2	27.2	28.82	38.35	45.72	52.2
Total Range	4.68 to 14.4	7.2 to 21.6	12.6 to 25.2	15.2 to 36.36	19.8 to 43.92	23.4 to 52.2	45.7 to 45.92	52.2
Mean Breadth	3.39	4.0	3.79	4.0 (4.09)	4.0 (4.09)	4.0 (4.21)	5.0 (4.08)	5.4
Total Range	2.5 to 3.96	2.88 to 6.1	3.2 to 4.9	3.2 to 6.48	2.88 to 6.48	3.6 to 6.8	4.32 to 6.8	5.4

TABLE VII

*Measurement of spores taken directly from the diseased pupæ of Epipyrops*

Septation of spores	0	1	2	3	4	5	6	7
1 Mean L.	14.56	21.36	26.0	31.6	37.8	43.2	44	
2 Range in L.	10-16	14-30.0	16-32	16-44	32-44	34-52	34-52	
3 Measurement of spores in $\mu$ Mean B.	4.0	4.0	4.4	4.5	4.5	5.0	5.0	
4 Range in B.	2.8 4.2	2.8 4.2	3.0 6.2	3.0 6.4	3.0 6.4	3.8 7.0	3.8 7.0	
Percentage occurrence of spores	14.6	21.5	14.6	34	6.7	6.5	1.3	0.6

TABLE VIII

Table comparing the percentage Occurrence of septate spores, Mean length, Mean Range in length of spores of *Fusarium sp* from *Epiphytrops* and the Cultures, 1, 2 and *Fusarium moniliforme* var *subglutinans*

Septation	0 Sep	1 Sep	2 Sep	3 Sep	4 Sep	5 Sep	6 Sep	7 Sep
(A) Percentage occurrence								
1 Spores from diseased larva	14.6%	21.5%	14.6%	34%	6.7%	6.7%	1.3%	0.6%
2 Spores from Culture I	37.7%	2.3%	Trace	Trace				
3 Spores from Culture II	3%	6%	7%	52%	8%	23%	1%	Trace
(B) Mean length in $\mu$								
1 Spores from diseased larva	14.56	21.36	26.0	31.6	37.8	43.2	44	
2 Spores from Culture I	8.1	15.41	20.1	25.3				
3 Spores from Culture II	9.59	14.25	18.2	27.2	28.2	38.8	43.72	52
4 <i>F. moniliforme</i> Shield var <i>subglutinans</i>	9	17		32		50		
(C) Mean range in length in $\mu$								
1 Spores from diseased larva	10-16	14-30	16-32	16-44	32-44	34-53	34-53	
2 Spores from Culture I	2-16	8-24	10-23	20-32				
3 Spores from Culture II	5-14	7-22	13-25	16-36	20-44	23-52	45-52	
4 <i>F. moniliforme</i> Shield var <i>subglutinans</i>	7-12	14-20		25-48		43-53		

The stroma is purple or pink in culture 1 (Ridgeway, 1912), while it is uniformly salmon or salmon buff in culture 2. The cultures agree closely with each other regarding the production and the characters of the microconidia. They are found generally thinly scattered on the agar surface or occasionally grouped together in false heads, predominantly continuous ovoid to spindle shaped, occasionally 1-3 septate, thin, straight or slightly curved with rounded apex, with bluntly pointed or slightly foot-celled base, the septations are indistinct and hyaline (Text-fig B).

Abundant conidia are found in the aereal mycelium. The conidia are not produced in chains.

In addition to possessing microconidia as above described, culture 2 is also characterised by the production of predominantly septate spores. The spores produced in aereal mycelium are 0-5 septate, rarely 6-7 septate, rod to spindle-shaped, with blunt ends or ends tapering to a blunt point, or with the ends slightly curving and tapering, without foot-celled base or rarely with distinct or indistinct foot-cell (Text-fig C).



Fig. A-C.—Fig. A. Conidia of *Fusarium moniliforme* Shield var. *subglutinans* Wenzl & Mig. from pupa of *Epipyrops* ( $\times 400$ ) Fig. B. Conidia of above from culture 1, producing only microconidia ( $\times 600$ ) Fig. C. Conidia of above from culture 2, producing conidia as in nature ( $\times 400$ ).

Chlamydospores are absent in both the cultures

The conidia from the mycelium of the parasitised *Epipyrops* are closely similar to those described under culture 2 (Text-fig A)

#### TAXONOMY

According to Wollenweber and Reinking's key for the identification for the groups and sub-groups of the genus *Fusarium* the abundant microconidia and lack of macroconidia in aerial mycelium and of chlamydospores, and nature of colours, place culture 1, in "Liseola"

Microconidia are not in chains Blue sclerotia are absent If the absence of blue sclerotia is recognized as a variable characteristic as Wollenweber (1935) does in the description of *F. moniliforme* Sheldon, then the other characters closely approximate to the above fungus Further, the absence of conidia in fast chains brings it to *F. moniliforme* (Sheldon) var *subglutinans* Wr and Rkg If the production of blue sclerotia is given more importance in distinguishing species, the fungus is brought down to *F. neoceras* Wr et Rkg But the spores are not as long as those of *F. neoceras*, being little more in fact than half the length Regarding culture 2, and the fungus from parasitised *Epipyrops* the spores in the aerial mycelium are not those of "Liseola"

The spore characters place the latter in the section, "Lateritum" which is after all very close to "Liseola" and overlaps it as seen in the Key of Wollenweber (also Padwick, 1941) The key characters employed to distinguish the two sections is the production of microconidia in chains in the latter and their being not in chains in the former In *F. moniliforme* v *subglutinans* the conidia are not in chains, but the fungus belongs to "Liseola" and is described under the section by Wollenweber

The presence of septate spores instead of microconidia raises an important issue Wollenweber (1935) states that, "*F. moniliforme* is variable and occurs in forms which oscillate in the septation of the conidia, sometimes suddenly rising to develop highly septate sickle-shaped spores in sporodochia and pionnotes far surpassing the normal in number, then further relapsing to produce mostly the microconidia" Further, according to Subramaniam and Chona (1938) the fungus isolated from sugarcane suffering from 'wilt' in Bihar and identified as *Chephalosporem sacchari* Butler by Mcræ produced abundant macroconidia typically like those of *F. moniliforme* in Holland When the cultures were received in India, they produced only microconidia Thus the variability in spore production of this fungus recorded in this paper is in conformity with the earlier observations mentioned above.

In comparing the data presented in Tables I and II, it will be seen that the two cultures agree closely with each other in the amount of growth, colour and amount of conidial production in all media except oatmeal and in the remarkable similarity in appearance and measurements of the 0-3 septate spores in Oatmeal, rice, and Brown's standard agar. In the above three media culture 2, produced mostly 0-3 septate spores.

Thus the fungus isolated from *Epipyrops* is identified as *F. moniliforme* Sheld. var. *subglutinans* Wr. and Rkg. [*Gibberella Fujikuroi* (Sawada) Wr. var. *subglutinans* Edwards].

*Parasitism of F. moniliforme var. subglutinans on Epipyrops*

*Experiment I*—Ten adults and nymphs of *Pyrilla* with *Epipyrops* larvæ were collected from the field, preserved in wire gauze chambers and fed on fresh sugarcane leaves. Using a small atomiser the parasites along with the hosts were sprayed upon with spore suspension from a fresh 20 days old culture of *F. moniliforme var. subglutinans*. The larvæ were not affected in any way even at the end of a week.

*Experiment II*—Specimens of pupæ attached to the sugarcane leaves were collected and sprayed upon with spore suspension of the above fungus as in experiment I. A profuse salmon coloured mycelium developed on the pupæ within 48 hours. The fungus was re-isolated and identified as *F. moniliforme var. subglutinans*.

The fungus is a parasite of *Epipyrops* in its pupal stage only. It is not able to parasitise the larva. In nature also only pupæ have been found attacked by the fungus.

#### SUMMARY

1 A species of *Fusarium* was found parasitising pupæ of *Epipyrops* which in its larval stage is a parasite on *Pyrilla*, a pest of sugarcane.

2 A remarkable variability between the isolates of the fungus was noticed. One set of isolates produced only microconidia in culture, while the rest of the cultures produced both micro- and macro-conidia. In the natural state both the micro- and macro-conidia were present.

3 The morphological features are given in detail.

4 The conidial character brings the culture producing only the microconidia nearest to *F. moniliforme* (Sheld.) var. *subglutinans* but blue sclerotia, however, are absent. But as this is stated to be a variable character in literature the fungus is regarded as *Fusarium moniliforme var. subglutinans*.

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# STUDIES ON SCLEROTIUM-FORMING FUNGI

## I *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

### Part 1 Cultural Studies

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### INTRODUCTION

A NUMBER of workers have attempted to correlate restriction of parasitic fungi to particular host plants with certain biochemical relationships, for example, with toxic properties of plant juice and with the capacity of fungi to secrete the pectinase enzyme. The primary object of the investigations in this series is to explore the position with regard to certain sclerotium-forming fungi. In the first instance the fungi selected, *Sclerotium cepivorum* and *Sclerotium tuliparum*, were chosen as being similar in habitat, in the type of plant part attacked, and to some extent at least in morphological features. The first step in the investigation was to determine how far these two fungi were restricted to their particular hosts, onion and tulip, respectively. That being established, the problem was then to try to explain the basis of the specialisation shown.

While the central idea was as outlined above, the investigation naturally followed a variety of lines, leading to a detailed cultural study of the two organisms concerned.

### HISTORICAL

The literature dealing with the fungi *Sclerotium tuliparum* Klebahn and *Sclerotium cepivorum* Berk is rather extensive. In the following account each fungus will be treated separately, in the order named.

The bulb-rot of tulips was long known in Holland and Germany where it caused severe damage. It was reported from Holland at least as early as 1884 and Wakker<sup>1</sup> appears to have been the first to describe the disease which he designated merely as the "tulpenziekte". He has given extraordinarily clear and accurate symptoms of the disease and the characters of the pathogen.

Ritzema Bos<sup>2,3</sup> describes a disease which was very destructive at the time in certain parts of Holland and which from the symptoms given was obviously the gray bulb rot. However, there was a certain amount of

confusion in his account with the blight due to *Botrytis* ("fire") He states that diseases of Iris, Hyacinth and Gladiolus due to the same *Sclerotium* also occur

Klebahn<sup>10 11 12</sup> in his earlier papers made the same confusion between two tulip diseases but later recognised them as distinct He is responsible for the name *Sclerotium tuliparum*, and he showed that the same fungus attacks a large number of hosts, in particular *Iris hispanica*, Hyacinth, *Fritillaria imperiales*, Yellow Narcissus, *Scilla sibirica*, *Galanthus nivalis* and *Crocus vernes*

Muller-Thurgau<sup>17</sup> and Lendner<sup>18</sup> reported the disease from Switzerland The latter observed that the newly formed bulbils may also be attacked

Whetzel and Arthur<sup>14</sup> have given a good historical resumé and have studied the taxonomic relationships of the fungus They say that the first indications of the disease are the bare spots in the tulip beds in the spring Nearly all bulbils in the soil contaminated area are usually so injured that they fail to grow When affected bulbils do send up leaves, their growth is greatly retarded, and they soon die and wither away Initial infection evidently occurs in the fall and early winter shortly after the bulbils are put out into the beds, or early in the spring When diseased bulbils are dug up, they are found to be more or less rotted, the infection being usually at the tip, or nose, of the bulb The healthy white tissue is turned to a grayish or a reddish gray colour The soil clings to the exterior of the rotted parts and embedded in the soil or in the rotted bulbils are sclerotia Experiments showed that the pathogen depends upon its sclerotia to tide over from one season to the next Mycelium, which is readily produced from the sclerotia, spreads through the soil and attacks the suspect The pathogen appears to be a low temperature parasite

Whetzel and Arthur remark that certain distinctive features in the morphology (especially in sclerotial structure and mycelial characters) of the pathogen show taxonomic relations to *Rhizoctonia solani* and *Corticium stevensii* They regard these as sufficient to warrant its transfer from the genus *Sclerotium* to *Rhizoctonia*

Brooks<sup>8</sup> gives an account of the disease of tulips and *Iris reticulata* caused by *Sclerotium* (*Rhizoctonia*) *tuliparum* Tulips or other bulbous plants affected by this disease may be either completely destroyed below the soil level, or they put forth shoots which appear above the ground but are dwarfed and malformed and never flower The sclerotia of the fungus cling to the neck of the bulb and the part of the shoot below soil level The infection almost invariably proceeds from the soil by the formation of

strands of mycelium from sclerotia already therein. He found that Hyacinths, Daffodils, *Scilla sibirica*, *Fritillaria imperialis* and *Iris hispanica* are also attacked.

Dowson<sup>18</sup> reports that *Sclerotium tuliparum* sometimes attack tulips and *Iris reticulata* in England causing gray bulb rot. He gives the usual symptoms of the disease. Infection is entirely due to contaminated soil and takes place in early winter. The parasite spreads but slowly from one place to another and is probably introduced into a new locality by a few small sclerotia embedded between the scales of otherwise perfectly sound bulbs.

Van Beyma Thoe Kingma<sup>28</sup> notes the frequent association of *Sclerotium tuliparum* with *Penicillium corymbiferum* on tulip bulbs, both of these being active parasites. Weber<sup>48</sup> gives the symptoms, etiology and control of sclerotial disease of tulips caused by *Sclerotium tuliparum*. Kawamura<sup>19</sup> states that tulip bulbs in Japan are liable to infection by *Sclerotium rolfsii* with which *Sclerotium tuliparum* is believed to be identical. Buddin<sup>11,12</sup> reports that *S. tuliparum*, besides attacking tulips, also occurs though generally less severely on Iris, *Scilla*, *Crocus*, *Ixia*, *Fritillaria Colchicum*, Hyacinth and Narcissus. Observations showed that bulbs planted with one half to two-thirds of their surface protruding mostly remained healthy even in badly diseased soil. Steaming of soil completely eliminated the disease. In the control of the disease when a powder containing chloronitro benzol was mixed with the surface soil 90 per cent control was obtained whereas sprinkling the soil after planting the bulbs was unsatisfactory.

For the first time in 1938 *Sclerotium tuliparum* was recorded in England and Wales on *Crocus*.<sup>49</sup> Osterwalder and Camenzind<sup>23</sup> tested 0.5 per cent formalin solution against *S. tuliparum* on tulips with satisfactory results.

Control measures, which rely chiefly on chemical disinfection of bulbs or soil, have been described by Caballero,<sup>13</sup> Wakker,<sup>41</sup> Ritzema Bos,<sup>26</sup> Klebahn,<sup>22</sup> Whetzel and Arthur,<sup>46</sup> Dowson,<sup>18</sup> Van Slogteren,<sup>44</sup> Buddin<sup>11,12</sup> and Osterwalder and Camenzind.<sup>23</sup>

White rot of *Allium* is a disease of widespread occurrence and was first recorded by Berkeley<sup>6</sup> in 1841 in Great Britain who named it *Sclerotium cepivorum* Berk. Voglino<sup>29</sup> recorded severe attack of leeks in Italy by *S. cepivorum* but on the basis of his cultural study he renamed the fungus *Sphacelia allii*. Cotton and Owen<sup>14</sup> reported that the white rot disease of onion bulbs caused considerable damage to onion crops in Great Britain. They found that shallots were markedly resistant and leeks did not appear to suffer. Caballero<sup>13</sup> reports considerable damage in garlic fields in Spain.

and regards *Sclerotium cepivorum* as the most destructive of the garlic parasites

Walker in a series of papers<sup>42,43,44</sup> described the white-rot of *Allium* caused by *S. cepivorum* in Europe and America and found that the disease occurred on onion Welsh onion leek garlic and shallot Leeks only suffered from the disease during cooler months The fungus attacked the plants at any time during the growing period provided external conditions were favourable He observed that the disease thrives best at moderately cool temperatures and with moderate soil moisture He found that within the temperature range favourable to growth of the plant, the fungus became less destructive as the rapidity of host growth increased

✓ Dowson<sup>15</sup> gives a brief description of *Sclerotium cepivorum* and its host He also observed that warm and damp weather favours the disease which is spread by the planting of diseased seedlings or sets and is increased by repeatedly planting onions in the same ground Nattrass<sup>28,29</sup> reports the occurrence of the disease from Egypt and Cyprus He describes its symptoms and says that overwintering is due to sclerotia in the soil and that the fungus did not grow above 30° C He further suggests that sets should only be planted from disease free areas and cultivation of the different species of *Allium* should be discontinued for 8 to 10 years

Du Plessis<sup>16,17</sup> gave a popular account of white mould on onion caused by *S. cepivorum* in South Africa and reports that disinfection of soil by formalin, heat or mercuric chloride are impracticable on a large scale though in laboratory test the sclerotia, which persist in soil and onion refuse for four or more years, succumbed to these treatments He found that losses may increase from 20 to 30 per cent when pink rot and bulb rot are accompanied by white mould caused by *S. cepivorum*

Onion varieties showing marked resistance to white rot have been developed at Manchester University<sup>45</sup> Matzulevitch<sup>46</sup> gives very brief description of the disease occurring in Russia From one locality in Czechoslovakia an epidemic outbreak of *Sclerotium cepivorum* on garlic is reported<sup>47</sup> Marchionatto<sup>48</sup> reports that *S. cepivorum* has been recognised since 1913 on onions and garlic in Argentina

Bremer<sup>9,10</sup> reports the presence of the disease in Germany and also recommends a well-regulated rotation in which onions are excluded from infested fields for at least 8 to 10 years He has also given a popular note on the rots of stored onions in Germany by *S. cepivorum* and other organisms Bremer and Nicolaisen<sup>19</sup> have given symptoms, etiology and control of the disease In New South Wales (Anon<sup>1</sup>) white rot has been recorded once on garlic and thrice on onions

Ogilvie and Hickman<sup>30</sup> found the disease widely distributed in Bristol province, mainly on white Lisbon spring onion. A soil application of a proprietary organic mercury compound in dust form containing hydroxy-mercurichlorophenol with 20 per cent organically combined mercury, before sowing, gave 56.8 and 17.9 per cent infection at two localities respectively against average of 86.7 and 90.4 per cent for the corresponding untreated control plots. Ogilvie, Croxall and Hickman<sup>31</sup> report that early autumn sowing of onions were more severely affected by white rot than were late sowings. Ogilvie and Walton<sup>32</sup> note that Up-to-Date Rousham Park Hero, Improved Reading and White Spanish onions are moderately resistant, leeks being only occasionally attacked.

Brandão<sup>7</sup> has given the symptoms and control of white rot affecting garlic in Brazil. The disease has also been recorded in Argentina by Hauman-Merck,<sup>18</sup> in United States by Valleau,<sup>37</sup> in Holland by Van Poeteren<sup>38</sup> and in various parts of Australia.<sup>1</sup>

Asthana<sup>3</sup> states that high potash manuring in England showed some decrease in the attack of *S. cepivorum* on onions but there was indication from plot experiments that liming reduced considerably more the incidence of the disease on onion seedlings. Moore<sup>36</sup> reports the fungus to be seed-borne and is usually transmitted by infected seedlings. Boorer<sup>2</sup> found that application of 4 per cent mercurous chloride (calomel) dust to the seed drill at sowing time gave better results than seed treatment. One lb of dust per 25 yd of seed drill gave good disease control in bulb onions, and one lb per 50 yd may suffice for salad onions.

#### MATERIAL AND METHOD

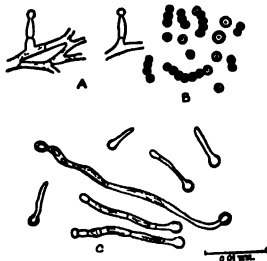
All through the experimental work pure cultures of *Sclerotium cepivorum* and *Sclerotium tulparum* were used. Isolations were made from diseased onion and tulip bulbs and the purity of all the cultures was assured by the single hyphal tip method of Brown.<sup>6</sup> All the cultures were maintained on potato-dextrose agar. Throughout the laboratory and field experiments "White Spring Lisbon" onion seeds, English onion bulbs and "Prince of Austria" tulips were used. The use of other varieties of onion and tulip will be mentioned at the appropriate places. Field and pot culture experiments were carried out at Slough and at Chelsea Physic Garden respectively.

#### MORPHOLOGY

Mycelial and sclerotial growths of *Sclerotium cepivorum* and *Sclerotium tulparum* were made on potato-dextrose agar plates. *S. cepivorum* forms a fluffy white mycelial growth, which is rather coarse with large cells. The

branches often anastomose and hold the hyphæ together in sheets or strands. The growth of the fungus is quite vigorous. After ten days of incubation at 20° C., sclerotia first appear as small white tufts of loosely intertwined branches. They become circular in form and within one to two days change into dull green from white. The outer layers darken and after a further two days the sclerotia appear as hard black bodies of 0.6 to 0.8 mm diameter. Within two weeks of inoculation they are formed in large numbers over the whole surface of the plate (Plate III, Fig. 1). In section the sclerotia show a black cortex and inside a medulla of elongated closely packed hyphæ, in this respect resembling the sclerotium of *Sclerotinia sclerotiorum*.

The only spores produced by *S. cepivorum* were microconidia. Voglino\*\* in the cultural study of the organism has described the production of sporodochia of hyaline conidiophores upon which were borne spherical, hyaline and catenulate conidia. Presumably the conidia of Voglino were the microconidia. It was observed that the microconidia were produced on certain media only, e.g., six week old plates of Brown's Starch agar and three to four week plates of tulip agar. These microconidia are formed on small conidiophores, 6–10  $\mu$  long and are spherical 2.5  $\mu$ –3.4  $\mu$  in diameter, with two walls. Very often they occur in chains, 2–8 sticking together, which arise by successive constrictions of the conidiophores. Attempts to germinate these microconidia almost uniformly failed. In a very few cases, about 14 spores altogether short septate germ tubes appeared but these soon cut off a microconidium at the tip and ceased to grow. The germination was observed only on 20 per cent Tulip juice (Text-Fig. 1).



TEXT-FIG. 1. Microconidia of *Sclerotium cepivorum*. A—Formation of microconidia, B—Microconidia. C—Germinating microconidia.

The hyphæ of *Sclerotium tuliparum* are long, slender and septate, the individual cells tending to be barrel-shaped when young. The mycelium grows rhizomorphically at a fairly uniform rate. It is mostly white, at first appressed and somewhat silky, later becoming more distinctly aerial towards the periphery. With age the colour of the mycelium gradually changes from white to clay. The medium soon becomes discoloured, taking on a distinctly reddish-brown tinge which deepens with age. The sclerotia first appear after 10 days as irregular, white, cottony masses on the surface of the culture, in a broad ring near the periphery of the colony (Plate III, Fig. 2). They soon turn to a pale yellow, deepening to reddish-brown, and becoming almost black when dry. They are generally globose to oblong bodies, 3–4 mm. in diameter but they vary a lot in their size and form with different media. In many cases several sclerotia are agglomerated into a large, irregular mass and in others the size varies from 1.5 to 8 mm. in diameter. In contrast to that of *S. cepivorum*, the surface of the sclerotium is dull, rough and irregular. In cross section the medulla is seen compact and definite in form, having globose cells.

#### GROWTH

*Nutrient Media*—Both the fungi under study were grown on 28 different natural and synthetic media. Petri dishes of an equal depth were poured, inoculated at the centre with *S. cepivorum* and *S. tuliparum*, and incubated at 20° C. for two weeks. The results of the comparative study of the mycelial and sclerotial growths are given in Table I.

Table I shows that both fungi grow more or less freely on a large variety of media and that in general the richer the medium the greater the mycelial and sclerotial development. *S. cepivorum* growth is favoured by an acid medium whereas *S. tuliparum* prefers a neutral or alkaline one. A representative set of growth form is illustrated in Plate III, Figs. 1 to 4 and Plate IV, Figs. 5–6.

Throughout the series the mycelium of *S. cepivorum* is white and woolly while that of *S. tuliparum* is clay coloured and appressed. Characteristic features of *S. cepivorum* and *S. tuliparum* respectively are the peculiar sweet musky odour in all the cultures and the discolouring of the medium which takes a reddish-brown tinge with age.

To a certain extent the number of sclerotia produced on the same medium varies with the depth of pouring. Table II gives the number of sclerotia of *S. tuliparum* per plate as counted by the naked eye while in the case of *S. cepivorum* the numbers refer to a standard microscopic field as the sclerotia are minute,

TABLE I

Comparative study of the mycelial and sclerotial growths on a variety of nutrient media

Media	<i>S. cepivorum</i>		<i>S. tuliparum</i>	
	Mycelial Growth	Sclerotial Growth	Mycelial Growth	Sclerotial Growth
Potato extract	++	++	++	++
Potato mush	+++	+++	+++	++
Turnip agar (20%)	+++	+++	+++	+++
Tulip agar (20%)	++++	++++	++++	++++
Pea agar (20%)	+	+	+++	++++
Oat meal agar	+	+	++	+
Malt agar	+	+	+	+
Prune agar	++	++	+	+
Lettuce agar (20%)	++	++	++	++
Leek agar (20%)	++	+++	+	+
Onion agar (25%)	++	+++	++	++
do (20%)	+++	+++	++	++
do (15%)	++	+++	++	+
do (10%)	++	++	+	+
do (7.5%)	++	++	+	+
do (5%)	+	+	+	nil
do (2.5%)	+	+	+	nil
Brown's Starch agar	+++	+++	++	++
Asparagin glucose	+	+	++	+
Acid Asparagin glucose	+++	++	+	+
Glucose peptone	++	++	+	nil
Glucose nitrate	++	++	+	+
Glucose $\text{NH}_4\text{NO}_3$	++	++	++	+
Glucose $\text{NH}_4$ tartrate	++	+	+	nil
Cane sugar nitrate agar	++	+	+	+
Coon's agar	+	+	+	+
Chohn's nutrient agar	++	+	+	+
Richard's agar	++++	+++	++++	+++

[ + = Scanty or a few ++ = moderate +++ = good ++++ = abundant  
sclerotia and thick mycelial growth + | + + + = luxuriant mycelial growth, large and  
abundant sclerotia ]

TABLE II

Amount of medium per stand nil plate	Number of sclerotia of <i>S. tuliparum</i> after 2 weeks		Number of sclerotia of <i>S. cepivorum</i> per microscopic field after 2 weeks	
	Mature	Immature	Mature	Immature
20 cc	136	19	25	12
40 cc	300	47	50	20

✓ Here it will be seen that with the depth of the medium (Brown's Starch agar) the number of sclerotia is increased in both the fungi while increase

in size was only observed in case of *S. tuliparum*, no such effect being produced on the sclerotia of *S. cepivorum*

Similarly the depth of plating influences the rate of linear growth. The figures in Table III give the increase in growth on Brown's Starch agar from the 4th to the 8th day. The effect is marked in the case of *S. tuliparum*, but negligible in the other.

TABLE III

Amount of medium per standard plate	<i>S. tuliparum</i>	<i>S. cepivorum</i>
10 cc	1.6 cm	3.6 cm
20 cc	2.3 cm	3.8 cm
40 cc	3.4 cm	4.2 cm

By referring to Table I it will be observed that media prepared from onion extracts were more favourable to the growth of *S. cepivorum* than to that of *S. tuliparum*. This point was investigated in greater detail.

The juice of onions and of tulips was squeezed out under a hand press, filtered through muslin and centrifuged to remove the coarse particles. Various dilutions of those extracts were then made and drops placed on cover slips in Ward-cells or on slides in moist petri dishes. These petri-dishes contained a layer of agar to which 0.4 per cent mercuric chloride was added and the slides were laid on this. By this method a moist atmosphere was maintained and the development of contaminating fungi and bacteria was reduced to a minimum. Each nutrient drop was inoculated with a hyphal tip of *S. cepivorum* or *S. tuliparum*. Table IV records the state of growth after 24 hours at 20° C.

TABLE IV

*Mycelial growth in concentrations of crude onion juice*

Fungus	Percentage of concentration of crude onion juice					
	10	20	40	60	80	100
<i>S. cepivorum</i>	++	+++	++++	+++	++	+
<i>S. tuliparum</i>	nil	+	nil	nil	nil	nil

[ + = scanty, ++ = moderate, +++ = good and thick, ++++ = luxuriant ]

The main point brought out is that *S. tuliparum* does not grow on any of the dilutions of crude onion extract (pH 6.0). The same was true after

60 hours It is noteworthy that *S. cepivorum* is also considerably affected in its growth at the higher concentrations of the extract

When however the onion extract was steamed for half an hour before use, the differential effect shown in Table IV was much lessened

TABLE V  
*Mycelial growth in concentrations of steamed onion juice after 24 hours*

Fungus	Percentage of concentration of steamed onion juice					
	10	20	40	60	80	100
<i>S. cepivorum</i>	++	+++	+++	+++	++++	++++
<i>S. tuliparum</i>	+++	++	++	++	+	+

(Notation as in Table IV)

*S. tuliparum* grew quite well on this medium, especially at the lower concentrations, and the retarding effect of high concentration on the growth of *S. cepivorum* also disappeared

That the effect of boiling was the dissipation of an inhibitory volatile substance was shown by studying the growth of the two fungi in turnip extract (20 per cent) in the presence of crude unboiled or boiled onion extracts. These experiments were carried out in hanging drops, the turnip extract with mycelial tip being on the cover slip, and the bottom of the cell containing the onion extract. The comparative results are shown in Table VI

TABLE VI  
*Mycelial growth after 24 hours on turnip extract in the presence of crude and boiled onion extracts*

Fungus	Crude onion extract	Boiled onion extract
<i>S. cepivorum</i>	+	+++
<i>S. tuliparum</i>	all	++

(Notation as in Table IV)

The corresponding results with tulip juice, (a) unboiled (pH 5.8), (b) boiled (pH 6.2) are shown in Table VII (Notations as in Table IV). On boiling the juice a precipitate was formed which was removed and the growth of the two fungi was observed in the clear filtrate.

TABLE VII

*Mycelial growth in different concentrations of tulip juice after 48 hours*

Fungus	Percentage of concentration of tulip juice					
	10%	20%	40%	60%	80%	100%
<i>S. cepivorum</i>	++	+	Unboiled juice nil	nil	nil	nil
<i>S. tuliparum</i>	++++	+++	+++	++	++	+
<i>S. cepivorum</i>	+	+	Boiled juice nil	nil	nil	nil
<i>S. tuliparum</i>	++	+++	+++	++	+	+

In contrast to the results with onion juice boiling has no obvious effect in making tulip juice more suitable for the growth of *S. cepivorum*

The conclusions arising from Tables IV-VII are that crude onion juice is highly inhibitory to the growth of *S. tuliparum* but that this inhibition is removed by boiling. On the other hand tulip juice is relatively unfavourable for the growth of *S. cepivorum* and this effect is not removed by boiling.

The fact that *S. cepivorum* and *S. tuliparum* prefer the extracts of their particular host plants is also shown, though not so markedly as in Tables IV-VII, by studying their growth rates on agar media compounded with these extracts. Comparative data after 48 hours are given in Table VIII.

TABLE VIII

*Growth rates in cm. on different concentrations of onion and tulip agar media after 48 hours*

Percentage of concentration of the medium	<i>S. tuliparum</i>		<i>S. cepivorum</i>	
	Onion	Tulip	Onion	Tulip
2.5	1.0	3.0	2.9	2.8
5.0	1.4	2.9	3.4	3.2
10.0	1.6	3.4	4.0	3.0
15.0	1.7	2.7	4.7	2.7
20.0	2.1	2.4	5.1	3.0
25.0	1.8	2.2	4.8	3.0
30.0	1.3	1.8	3.8	2.9
35.0	1.1	2.0	3.2	2.8

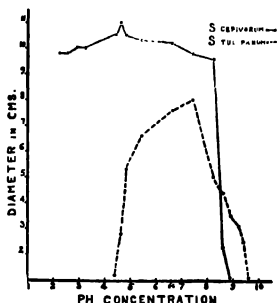
Here it is seen that each fungus grows somewhat more rapidly on an agar medium prepared from the juice of its own host plant.

Table IX, which gives the growth of *S. cepivorum* and *S. tuliparium* after 10 days at 20° C. on 20 per cent. extracts of the juice of Spanish and English onions, Spring onion leaves and Spring onion bulbs further illustrates the relatively slow growth of *S. tuliparium* on onion media

TABLE IX  
Growth of the colonies in cm on different onion media

Fungus		Spanish onion	English onion	Spring onion leaves	Spring onion bulbs
<i>S. cepivorum</i>	--	8.0	7.8	6.5	5.4
<i>S. tuliparium</i>	--	2.8	2.7	2.3	1.9

**H-ion concentration.**—The two fungi were grown on plates of Brown's Starch agar (pH 6.6). The medium was adjusted to different pH values by adding malic acid or sodium bicarbonate, which were separately autoclaved and added just before pouring. Inoculations of the plates were made in the centre by a single sclerotium in the case of *S. tuliparium* and three in case

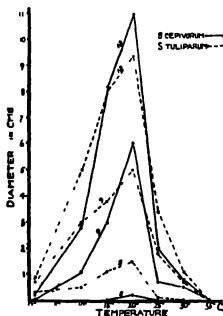


Text-Fig. 2. Illustrating growth of *S. cepivorum* and *S. tuliparium* at different H-ion concentrations.

of *S. cepivorum*. All the plates were incubated at 20° C. for 12 days when the colony growths were measured. The results are shown in Text-Fig. 2, in which each reading is an average of ten diameters from five different plates,

It will be seen from the figure that the growth of *S. cepivorum* is fairly constant over a wide range (2.2–8.2) of H-ion concentration. A higher pH than 8.2 causes the growth rate to fall considerably. On the other hand the curve for *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum*, and conversely for the acid side.

**Temperature**—The temperature response of the two fungi on potato-dextrose agar over the range 1°–35° C is shown in Text-Fig. 3. Each point in the curves represents the average of ten measurements taken from five-fold series of plates on the 5th, 9th and 13th day.



TEXT-FIG. 3. Illustrating effect of Temperature on Growth of *S. cepivorum* and *S. tuliparum*.

The optimum temperature for both fungi is near 20° C, the minimum somewhere near zero and the maximum between 30° and 35° C. At the optimum temperature, *S. cepivorum*, though slower in beginning growth, rapidly out distances the other. At temperatures removed from the optimum, both above and below, *S. tuliparum* is the faster grower.

**Light**—The effect of light factor was tested on cultures growing on 20 per cent. onion agar, Brown's Starch agar, 20 per cent turnip agar and Richard's agar but no significant difference was observed either in growth rate or in the general appearance of the cultures.

## SUMMARY

1. Brief accounts of the literature dealing with the fungi *Sclerotium cepivorum* Berk. and *Sclerotium tuliparum* Klebahn are given.

2. Morphology of the two fungi has been described. The only spores produced by *S. cepivorum* are microconidia on certain media only. Attempts to germinate these microconidia almost uniformly failed as only 14 spores altogether germinated on 20 per cent. tulip juice.

3. A comparative cultural study of *S. cepivorum* and *S. tuliparum* showed that while both grew well on a great variety of media, nevertheless there was in the case of each a certain amount of specific reaction to the juice of its own host plant. In particular, crude onion juice is markedly inhibitory to the growth of *S. tuliparum*. Boiling of the juice largely removes this effect. *S. cepivorum* does not grow well in tulip juice, boiled or unboiled.

4. *Sclerotium cepivorum* is favoured by an acid reaction of the culture medium while *Sclerotium tuliparum* by a neutral or slightly alkaline reaction.

5. The temperature range of growth for both fungi is approximately 1°-35° C., with an optimum near 20° C.

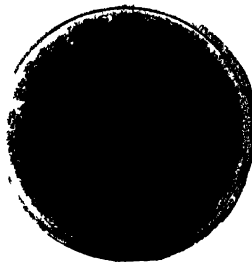
6. There is no significant difference by light factor either in growth rate or in the general appearance of the cultures of the two fungi.

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**F 1**



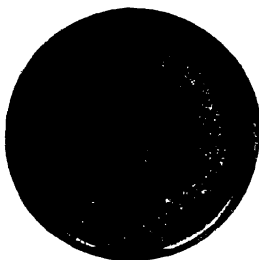
**Fe 3**



**Fe 4**



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## EXPLANATION OF PLATES

FIGS. 1, 3 and 5—Plate cultures of *Sclerotium cepivorum* on potato-dextrose agar, tulip agar and onion agar respectively.

FIGS. 2, 4 and 6—Plate cultures of *Sclerotium tuliparum* on potato-dextrose agar, tulip agar and onion agar respectively.

# STUDIES ON SCLEROTIUM-FORMING FUNGI

## I. *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

### Part 2 Symptoms, Mode of Infection and Host Range

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### INTRODUCTION

BULB-ROT of tulips and white-rot of onions were first reported by Wakker<sup>22</sup> in Holland and Berkeley<sup>3</sup> in Great Britain respectively. Bulb rot, caused by *Sclerotium tuliparum* Klebahn, was subsequently recorded in Germany by Klebahn,<sup>13</sup> in Switzerland by Muller-Thurgau<sup>18</sup> and Lendner,<sup>14</sup> in United States by Whetzel and Arthur<sup>24</sup> and Buddin,<sup>7</sup> in England by Brooks<sup>9</sup> and Dowson,<sup>10</sup> in Holland by Van Beyma Theo Kingma<sup>20</sup> and in Japan by Kawamura.<sup>12</sup> Similarly white-rot, caused by *Sclerotium cepivorum* Berk, was later reported in Italy by Voglino<sup>21</sup>, in Spain by Caballero,<sup>8</sup> in Europe and America by Walker,<sup>23</sup> in Egypt and Cyprus by Nattrass,<sup>19</sup> in South Africa by Du Plessis,<sup>11</sup> in Russia by Matzulevitch,<sup>16</sup> in Argentina by Marchionatto,<sup>15</sup> in Germany by Bremer,<sup>5</sup> in Brazil by Brandao<sup>4</sup> and in various parts of Australia (Anon,<sup>1</sup> 1938, 1943)

### SYMPTOMS AND MODE OF INFECTION

The earliest signs of attack of *S. cepivorum* on spring-sown seedling onions usually become noticeable about the end of May or beginning of June. The older leaves first turn yellow, starting from the tips downwards, and then fall over. The inner leaves collapse later on. Affected plants can readily be pulled from the soil, because the stem base is more or less completely rotted. Around the base of affected bulbs a white, fluffy mycelial growth is frequently seen. Later on sclerotia are formed on or inside the scales as the mycelium penetrates into the interior and gradually destroys the base and the scales so that the bulb becomes rotten and worthless (Plate V, Figs 1-3). The fungus causes a semi water decay of the scales. The sclerotia formed are minute, black and hard. Attack is not limited to young seedlings, but one frequently finds large bulbs in the autumn showing early stages of attack.

Hyphae of the fungus can readily be demonstrated ramifying through all the disintegrated tissue, roots, stem and leaf bases.

When onion seed is sown in ground artificially contaminated by the fungus, *e.g.*, by placing the seed on pieces of the fungal mycelium in the ground, more drastic attack may be shown. In such a case a large percentage of the seedlings may fail to come above ground.

The fungus may be seed-borne, as reported by Moore,<sup>17</sup> but is usually transmitted by infected seedlings, and distributed locally by cultivation. Cotton and Owen<sup>8</sup> suggest that in all cases the roots are attacked before the bulbs, but in the writer's opinion this is not the case. Field observation shows from time to time bulbs with the appearance illustrated in Plate V, Fig. 4. In such a case the base of the bulb is entirely rotted though a large percentage of the roots are still not invaded. Unless special precautions are taken such a bulb when pulled will tear apart at the stem base, part of the latter being still anchored to the ground by the healthy roots.

Inoculation experiments carried out in the laboratory support the view that the stem base is the part of the bulb most susceptible to invasion. Healthy bulbs, with the leaves cut off were suspended after careful washing and surface sterilisation, in moist glass jars and inoculated either—(a) on the uninjured surface of scale leaves, (b) on the injured surface of scale leaves, (c) on the uninjured stem base at the point of emergence of roots; (d) on the uninjured root surfaces. Ten bulbs were tested in each lot. The results are indicated in Table I.

TABLE I

Rot on the uninjured surface of scale leaves	Rot on the injured surface of scale leaves	Rot on the uninjured stem base at the point of emergence of roots	Rot on the uninjured root surface
Nil	+ +	+ +	+

While the fungus appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The point of entry has been found to be the natural wound caused by the emerging root. It is to be noted that no attack takes place through the surface of the intact outer scales.

The symptomatology of "gray-bulb rot" of tulips due to *S. tuliparum* is somewhat different from that of the "white-rot" of onions by *S. cepivorum*. In the field the disease is first indicated in the spring by the failure of bulbs in certain patches to appear above ground. Bulbs which are less severely attacked may send up some distorted leaves but as a rule no flower is formed. When such plants are dug up, one finds that the rot is at the

base of the leaves, i.e., at the nose of the bulb and that the leaves are only attached to the bulb by a thin brown connection of rotted tissues. In the cases where bulbs fail to come up, one is struck by the fact that soil clings to the exterior of the rotted part. The roots of a badly infected bulb may be perfectly sound. The rot is of a dry type and the healthy white tissue turns to a reddish gray colour and becomes brittle. Sometimes brownish-black sclerotia are formed inside the bulb scales but more usually they occur in the soil adhering to the bulbs. In advanced stages of infection the mycelium forms a felty layer between the scales of the bulbs.

The fact that tulips when planted in infected grounds at the normal depth (about 5 inches) are attacked at different stages probably indicates that the base of the shoot is infectible over a considerable period of time. If however the bulbs are planted very shallow, so that their noses are just below ground level, the base of the shoot passes through the susceptible stage rather quickly. This is illustrated in Table II which gives the results of an experiment with potted plants.

TABLE II

(20 bulbs were planted and inoculated in each case)

Description of inoculation	No. of plants growing above soil level	No. of plants flowering
1 Control (uninoculated)	20	19
2 Bulbs inoculated at nose when shoots are 1 1/4 inches long	5	Nil
3 Bulbs inoculated at nose when shoots are 2 1/4-3 inches long	17	15

Under such conditions the base of the shoot presumably becomes harder with thicker cuticle than when the bulb is deeply planted.

Attempts in the laboratory to infect portions of the flowering stem above ground or the leaf-blades, wounded or unwounded, were uniformly negative.

Pot experiments indicated that the roots are not directly attacked by the fungus. Thus in one case 20 bulbs were planted over the fungal mycelium, while 20 others had the mycelium placed on the nose. Of the latter 5 only grew and 2 produced flowers, of the former 16 flowered. In all cases it was the shoot bases and not the roots which were attacked.

The experiments on influence of soil moisture and temperature on infection were mostly confined to the attack of *S. cepivorum* on white spring onion (Lisbon variety).

The soil, a mixture of medium loam, sand and leaf-mould, was autoclaved and dried down. The water-holding capacity of such a soil was found to be 46 per cent of the dry weight. Six batches of this soil were then adjusted to moisture contents of 100, 80, 60, 40, 20 and 10 per cent of water-holding capacity. These were placed in varnished earthen pots 6" in diameter and with a depth of soil of about two inches. The pots were placed under bell-jars and sufficient water added daily to each to keep up a constant weight. The results of two sets of such experiments are given in Table III when the seedlings had grown for eight weeks.

TABLE III

Percentage of soil moisture	Experiment I		Experiment II	
	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with <i>S. cepivorum</i>	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with <i>S. cepivorum</i>
100	65	40	70	38
80	67	25	66	20
60	80	12	47	15
40	28	2	32	5
20	12	10	18	12
10	Nil	Nil	Nil	Nil

These results indicate that *S. cepivorum* is able to attack onion seedlings over the whole range at which ready germination takes place. There is some suggestion also that the greatest development of the disease is near about 40 to 60 per cent soil moisture, higher or lower percentages reducing the disease.

The effect after six weeks of varied soil temperature is shown in Table IV. The experimental pots were placed in a range of green-houses. The temperatures were not under very strict control.

TABLE IV

(Number of seeds used was 90 in each case)

Temperature in Centigrade	Number of healthy plants growing		
	Control	<i>S. tuliparum</i>	<i>S. cepivorum</i>
25°-30°	21	18	18
15°-18°	74	72	24
8°-12°	65	66	32
2°-8°	55	53	46

The figures indicate an optimum of attack somewhere in the neighbourhood of 13°-18° C. At the higher temperatures, germination was very poor even in the controls, but there was relatively little attack. At the lower temperatures germination was good, and there was little attack.

The good agreement shown between the series which was uninoculated and the one which was inoculated with *S. tuliparum* indicates, as before, that this fungus causes no attack.

#### HOST RANGE

An experiment was set up in a green-house in which 20 tulip bulbs were planted early in December in pots, five per pot, (a) without addition of fungus, (b) with mycelium of *S. cepivorum* at top and bottom of bulbs, and (c) with mycelium of *S. tuliparum* placed as in (b). Observation five months later gave the results shown in Table V.

TABLE V  
(20 tulip bulbs were planted in each case)

Fungus	No. of plants grown	No. of plants flowering
Soil uninoculated	20	18
<i>S. cepivorum</i>	20	18
<i>S. tuliparum</i>	6	5

There was thus no evidence that *S. cepivorum* had produced any effect.

This experiment was repeated on a larger scale in the following year in the open ground. 12' x 12' area was divided into 12 rows, and in each row 20 Prince of Austria tulip bulbs were planted at a distance of six inches apart. The first row was uninoculated, the second inoculated by *S. cepivorum* at the top and at the base of the bulbs and the third similarly by *S. tuliparum*. This scheme was replicated four times so that altogether 80 bulbs were subjected to each treatment. All the bulbs were planted and inoculated in November. The number of plants which had come above ground were counted in March and the number of plants flowering were recorded after another two months. The data are given in Table VI.

It is thus clear that under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent infection, *S. cepivorum* had no ascertainable effect whatsoever. None of the bulbs inoculated with *S. cepivorum* showed any trace of the latter fungus, either on the scales or on the roots, at the time of lifting.

TABLE VI  
(80 bulbs were planted in each case)

Fungus	No. of plants appearing above ground	No. of plants flowering
Soil uninoculated ..	75	73
<i>S. cepivorum</i> ..	78	78
<i>S. tuliparum</i> ..	4	1

From laboratory experiments, it did not appear that *S. cepivorum* could attack tulip tissue even when the epidermis was removed. Some growth of the fungus took place but there was no obvious effect on the bulb tissue.

The behaviour of the two fungi towards onion plants was tested in a series of pot experiments, the results of which are set out in Tables VII and VIII. 80 seeds were sown in drills, with or without inoculation, in each case in the unautoclaved soil series while 108 in the autoclaved one. More extended tests which gave substantially the same conclusions were carried out on a field plot scale and the results are given in Table IX.

TABLE VII  
(Ten autumn-sown sets were planted in each case)

Treatment	Number of plants grown	Number of plants healthy
Uninoculated soil ..	10	10
Inoculated by <i>S. cepivorum</i> ..	6	4
Inoculated by <i>S. tuliparum</i> ..	9	9

TABLE VIII  
Percentage of growth

Treatment	Unautoclaved soil	Autoclaved soil
Uninoculated soil ..	72.0	83
Inoculated by <i>S. cepivorum</i> ..	43.7	30
Inoculated by <i>S. tuliparum</i> ..	67.0	76

On account of a certain amount of variation in the percentage germination of the seeds, the results are not so clear cut as in the converse case of tulip bulbs described above. The two tables however show definitely that *S. tuliparum* produces no attack of onion sets or seedlings under conditions

where *S. cepivorum* caused the loss of approximately 50 per cent of the plants

A comparison of the results of the Table VIII suggests that the capacity of *S. cepivorum* to attack is somewhat greater in autoclaved than in ordinary soil

TABLE IX

(400 onion seeds were used in each case)

Treatment	Percentage of healthy plants
Control (uninoculated)	43.0
<i>S. tuliparum</i>	48.0
<i>S. cepivorum</i>	25.0

It appears therefore from these experiments that neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other

A series of inoculations was carried out with *S. tuliparum* and *S. cepivorum* on a miscellaneous assortment of plants possessing bulbs, corms, etc. Inoculations were made on wounded or unwounded materials either in moist chambers or in the soil of pots. The following is a summary of the results obtained

(a) *S. tuliparum* caused 80-100 per cent infection of Single Early tulip (Artus), Single Tulip (Prince of Austria), *Scilla sibirica*, Hyacinth (Crimson), *Chionodoxa luciliae*, *Iris hispanica* (King of Whites)

(b) *S. tuliparum* caused infection in 40-60 per cent of Gladiolus (peach Blossom), Narcissus (*Poeticus ornatus*), Daffodil (Princeps), Crocus (Light Blue), Snowdrop (Single). Rhizomes of winter Aconite was less frequently attacked, only 20 per cent

(c) *S. tuliparum* produced no attack on English and Spanish mature onion bulbs, Spanish and English grown autumn sown sets, seedlings of white Lisbon and Red onion, Shallots, Leek (Musselburgh)

(b) *S. cepivorum* was not seen under any conditions to attack any of the plants listed under (a) or (b), whereas it vigorously attacked most of the onion types given under (c). Red onions, leeks and shallots were attacked to an extent of 20-25 per cent. only, i.e., less than the other onion types

It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts

The symptoms and mode of infection of both fungi on the above hosts are almost the same as described above for the natural hosts, i.e., *S. cepivorum*

attacks the base of the bulbs and *S. tuliparum* the top and young growing roots

## SUMMARY

1 Symptoms on the natural hosts and the modes of infection of *S. cepivorum* and *S. tuliparum* are described. While *S. cepivorum* appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The point of entry has been found the natural wound caused by the emerging root. In case of *S. tuliparum* it was the shoot bases and not the roots which were attacked.

2 *S. cepivorum* is able to attack onion seedlings over the whole range of soil moisture at which ready germination takes place—the greatest development of the disease being near about 40 to 60 per cent soil moisture. As regards temperature effect, the optimum attack is somewhere in the neighbourhood of 13° to 18° C.

3 Under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent infection on tulips, *S. cepivorum* had no ascertainable effect whatsoever.

4 Neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other.

5 *S. tuliparum* caused 80–100 per cent infection of Tulips, *Scilla sibirica*, Hyacinth, *Chionodoxa luciliae*, *Iris hispanica*, 40–60 per cent of Gladiolus, Narcissus, Daffodil, Crocus, Snowdrop, 20 per cent of rhizomes of winter Aconite, and produced no attack on onions, shallots and leek. *S. cepivorum* on the other hand attacked vigorously most of the onion types, leeks, shallots and red-onions only up to an extent of 20–25 per cent, while it could not attack the hosts of *S. tuliparum*.

6 It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts.

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## EXPLANATION OF PLATE

- FIG. 1. Two healthy spring onion bulbs with roots.
- FIG. 2. Two diseased spring onion plants. The black covering all round the base in the mycelial mat and sclerotia of the pathogen.
- FIG. 3. An advanced case of attack of *S. cepivorum*. The photo shows the whole plant of a spring onion, the leaves have all dried and fallen off.
- FIG. 4. An early stage of infection by *S. cepivorum* on onion bulb. Though the base of the bulb is rotted yet only a few roots have gone. A high percentage of healthy roots is shown here.

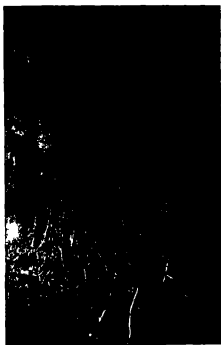


FIG 1



FIG 2



FIG 3



FIG 4



## STUDIES ON SCLEROTIUM-FORMING FUNGI

### I. *Sclerotium cepivorum* Berk and *Sclerotium tuliparum* Klebahn

#### Part 3 Pectinase Activity and Preparation

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THERE are certain factors which condition invasion of particular hosts by some fungi and not by others. The chief relevant references are given below.

Walker, Lindegren and Bachmann<sup>1</sup> report the presence of toxic substances in the juice extracted from succulent onion scales. They remark that these toxins are of two general types, one which is neither removed nor broken down readily by heat and one which is volatile and passes off from the extracted juice at room temperature within a few hours. There is a gradual decline during storage of onion bulbs in the amount of volatile toxin, a decline which is hastened by increase in temperature. The fungal spores generally become more sensitive with age to the volatile toxin. When comparing onion pathogens and non-pathogens they found no strict negative correlation between pathogenicity to onion and sensitiveness to the toxins, which only indicates that other factors enter into the determination of the parasitic relation. Considering the onion parasites as between themselves there was evident a negative correlation between aggressiveness of parasitic attack and sensitiveness to the dissolved and volatile toxins. The presumption, however, is that as the parasites invade the tissue the host toxins, though attenuated by fungus enzymes may possibly exert some retarding effect upon the invader. If this be the case, it is suggested that the host toxins may be one of the numerous factors which determine the degree of parasitism attained by a given parasite.

Vasudeva<sup>2</sup> gave an analysis of the factors responsible for the failure of *Monilia fructigena* to attack onion and *Botrytis Allii* to attack apple. He observed that the chief feature shown by spores of *Monilia*, when placed in wounds on onion, is their failure to germinate. This is due to the presence of a thermolabile substance which can be extracted with ether or chloroform. On the other hand, the failure of *B. Allii* to attack apple tissue is not due to any inhibitory or retarding action of apple juice. It could be made

to parasitise by adding to the inoculum a certain concentration of a nitrogenous substance. The effect of a nitrogenous compound in stimulating attack by *B. Allii* was found to run parallel with its effect in stimulating the secretion of the pectinase enzyme. He also found that by artificially ripening the apples, they become susceptible to *B. Allii* attack.

Chona<sup>2</sup> studied the enzymic behaviour of certain apple-attacking fungi (*Botrytis cinerea*, *Fusarium fructigenum*) with that of parasites on potato (*Pythium* sp., *Phytophthora erythroseptica*). Ordinarily the apple attacking fungi did not attack potato and *vice versa* but he found that with the supply of an additional nitrogenous food a certain amount of such cross infection could be brought about. The pectinase activity could be retarded by pH concentration and by the action of certain plant extracts and chemicals, the retardation depending on the medium into which the enzyme was secreted.

Menon<sup>3</sup> has shown that the behaviour of the pectinase is modified by the nutrient medium in which the fungus is growing. According to him the nature of the nutrient medium modifies the capacity of a fungus to secrete pectinase. He assumes that certain substances are adsorbed from the nutrient medium and this adsorption modifies the properties of the pectinase.

Thornberry<sup>4</sup> has dealt with the pectinase activity of eight strains of *Fusarium* sp. from tobacco stems, two of *Sclerotium bataticola* (*Macrophomina phaseoli*), *Sclerotinia sclerotiorum*, *S. trifoliorum*, *Rhizoctonia* sp. from tobacco, three strains of *Thielaviopsis basicola*, *Phytomonas* (*Bacterium*) *mori*, *P. tabaci* (*Bact. tabacum*) and *P. angulata* (*Bact. angulatum*). The determination of the pectinase activity was according to the method of Neuberg and Ostendorf (*Biochem. Z.*, ccxxix, p. 464, 1930). According to them extracts from pectase active plant tissues hydrolyse the ester linkage of the half calcium salt of monomethyl tartaric acid. The ester being water-soluble and hydrolysable by pectase into soluble methyl alcohol and insoluble half calcium salt of tartaric acid, this method of determining pectase activity offers promise of utility for quantitative measurements based upon the precipitate formed. Thornberry by working with the above method observed that freshly isolated cultures of *Fusarium* sp. gave moderate hydrolysis, whereas little or no activity was shown by those that had undergone repeated subculturing since removal from their host. *S. sclerotiorum* and *S. trifoliorum* were only slightly active but considerable hydrolysis took place in the tubes inoculated with *M. phaseoli*. The tobacco *Rhizoctonia* gave negative results, while those obtained with *T. basicola* were variable.

The pectinase enzyme of the two fungi, *Sclerotium cepivorum* and *S. tuliparum*, was prepared from cultures on plugs as well as on flasks,

Only the enzyme excreted by the fungi was taken into account. The enzyme by the plug method was prepared by placing blocks of potato, turnip, etc., in boiling tubes having absorbent cotton wool soaked with water, at the bottom. Such tubes were autoclaved, inoculated and incubated at 20° C for different periods. The decayed portion as well as the fungal growth was removed and the juice squeezed, centrifuged and tested for pectinase. To obtain pectinase from flask cultures, 40 c.c. of medium were inoculated in 500 c.c. conical flasks and incubated at 20° C for 10 to 20 days. The liquid was then filtered off and tested for pectinase. The test of activity was the usual one of the disintegration of potato discs (50  $\mu$  thick) as described by Brown<sup>1</sup>. For each experiment, as far as possible, potato discs were taken from the same potato so as to avoid tissue variation.

Preparations of the external enzyme were made in a standard manner from 15 days old plug cultures of potato, carrot, turnip, tulip and onion. These, when tested on standard potato discs, gave the activities shown diagrammatically in Fig. 1.

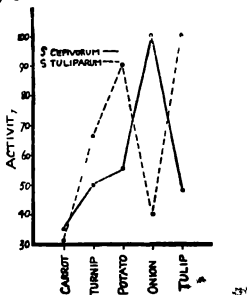


FIG. 1. Pectinase activity of *S. cepivorum* and *S. tuliparum* on different plug cultures.

From the figure it is clear that on media other than onion the activity of *S. cepivorum* is less than that of *S. tuliparum*. This applies also to tulip as a medium. On the other hand, the enzyme prepared from *S. cepivorum* on onion plugs is much stronger than that of *S. tuliparum* on this medium.

The corresponding diagram for the enzymatic extracts prepared from 15 days' old flask cultures is given in Fig. 2,

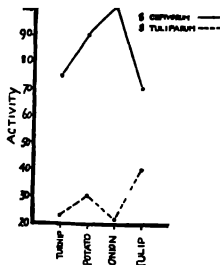


FIG. 2. Pectinase activity of *S. cepivorum*, *S. tuliparum* on flask cultures

It will be noticed that here in all cases the pectinase activity of *S. cepivorum* is much stronger than that of *S. tuliparum*, but the tendency of the curves is the same as in the previous figure

The enzyme from both the fungi was also obtained from 15 days' old flask cultures of synthetic and 20 per cent tulip and onion extracts. The pectinase activity on different cultures from the two fungi are given in Table I

TABLE I

Fungus	Pectinase activity			
	Richard's solution	Brown's starch	20% onion	20% tulip
<i>S. cepivorum</i>	100	71	83	80
<i>S. tuliparum</i>	100	83	71	86

The activities recorded in Figs 1 and 2 have reference to potato discs as test material. A comparative study of potato, tulip and onion discs gave the data shown in Table II in which the times required for disintegration are recorded.

A comparison of columns 2 and 5 of this table shows that *S. cepivorum* produces a more active enzyme than *S. tuliparum* when onion plugs are used as media and that the converse applies when tulip plugs are used.

TABLE II

Fungus	Pectinase from onion plug			Pectinase from tulip plugs		
	Potato discs	Tulip discs	Onion discs	Potato discs	Tulip discs	Onion discs
<i>S. cepivorum</i>	40 min	3 hrs and 30 min	1 hr and 50 min	60 min	2 hrs and 55 min	3 hrs and 10 min
<i>S. tuliparum</i>		3 hrs	3 hrs and 40 min	40 min	2 hrs and 25 min	3 hrs. and 20 min

Comparison of columns 2 and 3 shows that though the enzyme of *S. tuliparum* is only about half as active as that of *S. cepivorum* when tested on potato discs, it is fully more active when tested on tulip material. In other words tulip material is specifically more sensitive to the enzyme of *S. tuliparum*.

Similarly a comparison of columns 5 and 7 shows that the enzyme of *S. cepivorum* is specifically more active on onion material than is the enzyme of *S. tuliparum*.

*Effect of various factors on Pectinase activity*—The data as regards the effect of H-ion concentration on the pectinase activity are given in Fig 3

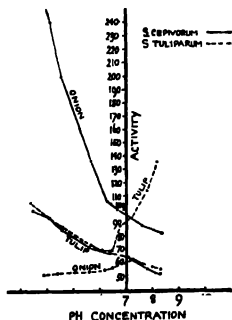


FIG. 3. Relation of H-ion concentration to the pectinase activity of *S. cepivorum* and *S. tuliparum*.

To obtain a range of pH, different amounts of N/20 HCl or NaOH were added. As a control to this experiment another range of pH was set up without the extracts which were tested for pectinase activity. Here it was found that within ten hours time there was no disintegration in the potato discs within 3.5–8.6 pH range. Therefore any effect shown within this range was due to the pectinase present. The pectinase activity of the extract without any added acid or alkali was taken to be 100.

A study of the above figure shows that there is a marked liking of *S. cepivorum* enzyme for acidity, especially the one from onion plugs. The curves for the preparation of *S. tuliparum* do not slope continuously to the right but either show no definite response to pH concentration at all or show a minimum of activity near the neutral point and a rather steep upward gradient on the alkaline side.

It has already been shown experimentally that the growth of *S. cepivorum* is fairly constant over a wide range (2.2–8.2) of H-ion concentration. A higher pH than 8.2 causes the growth rate to fall considerably. On the other hand the growth of *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum* and conversely for the acid side.

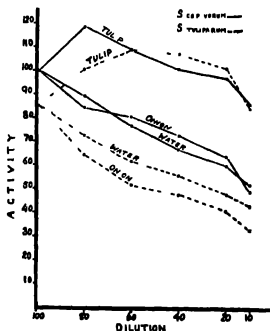


FIG. 4. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from onion plugs.

It is interesting to recall in this connection that *S. cepivorum* is favoured in its growth, as stated above, by an acid reaction, so that to that extent the reactions of the fungus and of its enzyme are similar

The relative retarding effects of various diluting substances (water, tulip or onion juice) are shown in Figs 4 and 5. In the former the medium used was onion and in the latter tulip plugs. The cultures in both cases were three weeks old

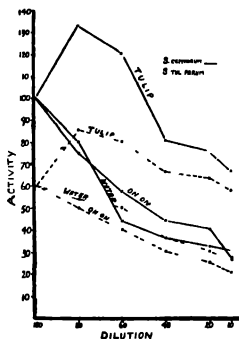


FIG. 5. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from tulip plugs

From both of these figures it is seen that certain dilutions by tulip juice instead of reducing considerably increase the activity while the presence of onion juice retards. The extracts of both fungi behaved similarly in this respect

#### SUMMARY

1 *Sclerotium cepivorum* and *Sclerotium tulparum* both were found to excrete pectinase enzyme on a variety of media

2 *Sclerotium cepivorum* gave more active preparations of this enzyme when grown on onion than on tulip tissue, and the converse was true for *S. tulparum*

3. There was evidence that tulip tissue was specifically more sensitive to the enzyme of *S. tuliparum* than to that of *S. cepivorum* and conversely.

4. The enzyme prepared from *S. cepivorum* was more tolerant of acidity than that of *S. tuliparum*.

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# STUDIES ON THE REFRACTIVE INDEX OF MILK

## II Some Factors Affecting the Refractive Index and Refractive Constant of Milk

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THE refractive index (R I) and Refractive Constant (K) of a large number of samples of cow and buffalo milk<sup>1</sup> and their normal limits of variation were given in an earlier paper (Rangappa, 1947). The samples in that experiment had been largely chosen at random from a big herd of about 400 animals without paying particular attention to any of the factors that are likely to affect the two values. But these values having been newly worked out for milk, the causes and extent of their variation under natural and routine conditions of animal management are to be looked for. With this object in view the effect of the more common factors like parturition, time of milking, quarter of udder, season processing, storage of milk, etc., have been studied in this paper.

### EXPERIMENTAL RESULTS

Milk samples were obtained from the same dairy farm which supplied samples for the last set of experiments (*loc cit*).

*Effect of Calving*—Calving began this year in June and lasted through July up to about the end of August. This happens to fall in with the rainy season which continues intermittently up to November. The R I and K of a number of samples were followed through the colostrum stage until the values became normal. Fig 1 illustrates the effect of this factor.

The high initial values, it will be seen from the figure, come down to a steady normal in less than 5 days. It may be mentioned that the initial values fall in with the high S N F contents of the colostrum. The refractive constant, also high initially, reaches normal levels a little sooner than the R.I.

*Diurnal Variations*—Individual and bulk samples (from 15 to 25 animals) analysed over a week are illustrated in Fig 2.

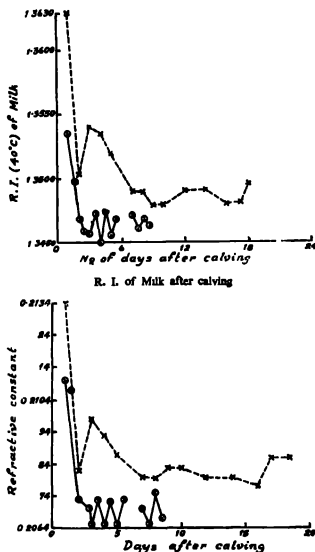


FIG. 1. Variations of Refractive Constant after calving

The figure shows appreciable fluctuations from day to day both of the R.I. and K of milk. But the variations in bulk samples are, as might be expected, not so wide as in individual samples. It is also to be noted that the range of variation of K is much less marked than that of R.I.

*Effect of Time of Milking.*—Fig. 3 illustrates the variations of the constants with the time of milking. The animals were milked at 7-30 in the morning and at about the same hour in the evening.

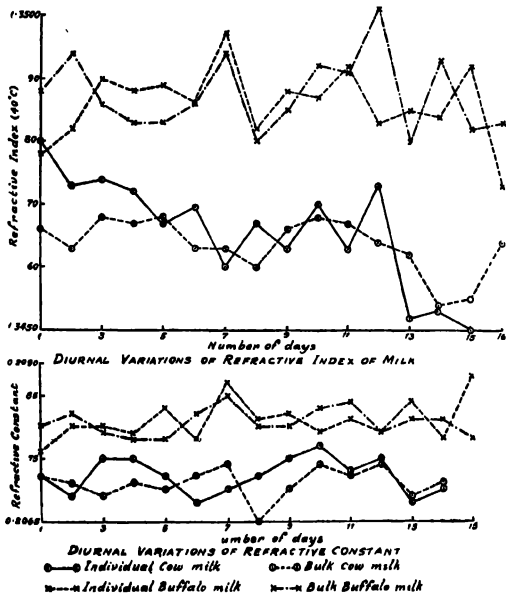
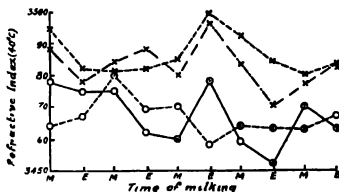


FIG. 2. These notations refer to all the figures in this paper

The figure shows that while the constants differ from morning to evening, the order of variation, like the differences from day to day, is unpredictable.



Variations in Refractive Index of Milk with Time of Milking  
M - Morning E - Evening

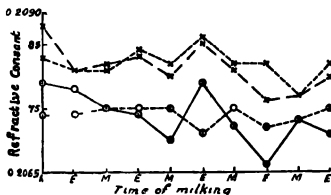


FIG 3 Variations in Refractive Constant of Milk with Time of Milking

**Effect of Season**—The climate of Bangalore being more or less, temperate throughout the year no violent seasonal changes in the form of extreme cold or extreme heat is experienced. The only difference marking one part of the year from another is the wet and dry seasons. In 1945 the rains ceased in November marking the commencement of the dry season. From December onwards the animals began to feed on dry fodder (ragi straw) for roughage which continued up to end of April 1946, the peak of summer or dry season. In May the heavy S W monsoon rains started and continued intermittently up to the middle of June. During this time the animals were given partly green and partly dry fodder. This was followed by the N E monsoon beginning from the middle of July with frequent rains which lasted (unusually) through November and December. In the short interval between the two monsoons the cattle were fed for a few weeks on dry fodder, after which (August to December), they were given roughage made up half

of greens (maize stock grass and lucerne) and half of chopped hay. The concentrates (groundnut cake, rice or wheat bran and Bengal gram) at the rate of 1 lb for every 3 lb yield of milk remained the same throughout the year.

Bulk and individual samples were analysed twice a week on Monday and Friday from January to December 1946. The monthly averages of R I and K of these samples are given in Fig. 4. In addition to these a large number of individual and bulk samples were also analysed every month. Table I gives the maximum and minimum values of R I and K of all the samples.

TABLE I  
Seasonal variations in the R I and K of Cow and Buffalo Milk

Season	Cow							
	Individual				Bulk			
	R I (40 C) Max Min		K Max Min		R I (40 C) Max Min		K Max Min	
January to April Average	1 3462 1 3443 1 34 4		0 2075 0 2065 0 2068		1 3470 1 3449 1 3455			
May to July Average	1 3470 1 3453 1 3459		0 2074 0 2065 0 2069		1 3470 1 3449 1 3459		0 2074 0 2065 0 2070	
August to December Average	1 3478 1 3450 1 3466		0 2080 0 2064 0 2072		1 3472 1 3458 1 3463		0 2075 0 2068 0 2071	
BUFFALO								
January to April Average	1 3488 1 3462 1 3474		0 2086 0 2077 0 2081		1 3488 1 3461 1 3477			
May to July Average	1 3492 1 3462 1 3479		0 2084 0 2076 0 2081		1 3484 1 3462 1 3474		0 2086 0 2076 0 2081	
August to December Average	1 3497 1 3471 1 3482		0 2088 0 2076 0 2080		1 3501 1 3468 1 3479		0 2088 0 2073 0 2079	

It can be seen from the table and the figure that the rainy season probably due to green feed causes considerable change in the order of values of refractive index of milk. From May onwards (except for the interval between the monsoons in July) to December and January there is a marked upward shift in the limiting values of the refractive index of milk especially of cows, thus setting different limits for the wet and dry parts of the year. This is noticeable in both individual and bulk samples. The buffalo how-

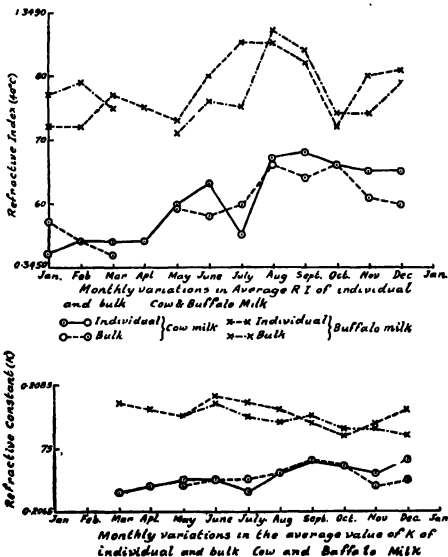


FIG. 4

ever, seems to be a little less susceptible to the changes in season than the cow. It should also be mentioned here that in normal years, when the rains usually end in November, the fall in R.I. might be expected to start earlier than during the current year. The refractive constant, on the other hand, runs more or less evenly within its narrow limits all through the year. This is obviously because the green feed which raises the R.I. also raises the density of milk.

**Portions of a Milking**—A number of individual animals were milked, from fore milk to strippings in 3 or 4 nearly equal parts examined for R I and K, and the examination repeated after pooling all the yield from the animal. An example of the type of variation of the two values are given in Table II

TABLE II  
*R I and K of Portions of a Milking*

COW		BUFFALO		
Portion	R I (40° C)	K	R I (40° C)	K
Fore milk	1 3487	0 2069	1 3477	0 2073
Middle	68	73	78	79
Final	67	78	78	82
Pooled milk	69	75	78	78

The table shows that only the refractive constant steadily rises from fore milk to strippings, and that the pooled milk gives values of K always within normal limits. This is accounted for by the falling density of the later portions of the milking (owing to the increasing fat content) while the R I remains practically the same.

**Different Quarters of the Udder**—Milk collected separately from the four quarters of the udder have also been analysed. The results are given in Table III.

TABLE III  
*R I and K of Milk from Different Quarters of Udder*

COW		BUFFALO		
Quarter	R I (40° C)	K	R I (40° C)	K
Left fore	1 3488	0 2067	1 3483	0 2079
Left hind	72	63	61	77
Right fore	69	68	67	76
Right hind	74	74	61	77
Bulked	70	75	68	83

The figures in the table show that appreciable differences do exist in the values of the constants of milk from the different quarters of the udder. But the differences are neither orderly nor predictable. Thus is to be expected as each quarter is a unit which functions independent of the others. The constants of pooled milk, however, lie within normal limits.

**Storage of Milk**—In commercial practice there is usually a time lapse between milking and retailing the milk to the consumer. The effect of this factor on the refractive index was therefore studied. Milk samples were stored at room temperature ( $17^{\circ}$ – $25^{\circ}$  C) in conical flasks plugged with cotton-wool. The acidity and R I tested every few hours until the milk finally curdled. The graph connecting acidity with R I is given in Fig. 5.

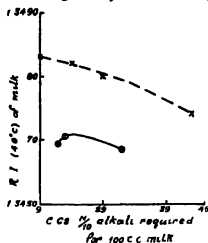


Fig. 5 Effect of Acidity on R I of Milk

It is apparent from the figure that the fall in R I is quite slow and gradual, there being a slight rise occasionally in the initial stages. Elsdon and Stubbs (1927) have also observed this fact with respect to milk serum. But the degree of or per cent variation in the R I of milk with the rise in acidity before curdling compared to the variation in the R I of serum from sour milk or of stored serum is very inconsiderable. And the disadvantage referred to by the above authors arising from the rise in the value of R I of serum of old milk or of stored serum fails to arise in the case of the present method wherein it is impossible to determine the R I of soured milk for the simple reason that it has ceased to be milk owing to the precipitation of one of the major constituents, the casein, of milk. But this limitation of the applicability of this method to fresh milk can be overcome by adding the minimum amount of formaldehyde necessary to keep the milk from curdling. For it has been shown by Schultz and Wein (1913) that addition of formalin in such minute amounts causes no palpable difference in the refractive index of the milk serum.

In terms of time of storage of milk, it has been found in this experiment that there is no detectable change in the R I in the first 8 to 12 hours of storage.

It may be mentioned here that in all these experiments the limits of R I and K of cow and of buffalo milk remain characteristically distinct from one another

**Effect of Processing**—Boiling is the popular method of processing milk in India. Two litre samples of milk were therefore boiled in an open tinned-brass vessel over a kerosene stove with continuous agitation (to prevent the formation of skin and residue) up to a total of ten minutes after the milk commenced to boil. At intervals of a few minutes the reduction in volume, the R I of the processed milk and the densities were determined. Fig 6

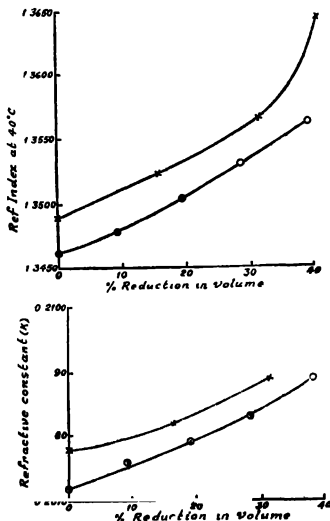


FIG 6. Effect of Boiling on R I and K of Milk

represents the order of variation of R I and K, with respect to fall in volume of milk. The initial and final composition of the milk are given in Table IV.

TABLE IV  
*Effect of Boiling Milk on the Refractive Index and Constant of Milk*

Milk	Density (20° C)	Fat %	S N F % (Calculated)	R I (40° C)	K
Unprocessed	1.0292	5.2	9.05	1.3468	0.2072
Boiled for 10 minutes	1.0456	6.1	13.76	1.3583	0.2141

It is evident from the above figure and table that the R I and K rise steadily with the rise in concentration of processed milk.

Finally, in order to facilitate the determination of the R I at the prevailing room temperature, readings were taken at 15°, 20°, 27°, 35° and 45° C. It was found that for every 1° C rise of temperature, the R I of milk falls by 0.00012 in the range between 20° and 45° C.

#### SUMMARY

A number of routine natural and artificial factors that are likely to affect the values of the refractive index and refractive constant of cow and buffalo milk have been investigated.

Colostrum exhibits a high R I and K, both of which reach normal levels in 3 to 5 days after parturition.

Differences in the two constants of milk occur from milking to milking, from day to day and between milks from different quarters of the udder. But the order of variation is unpredictable in every case. The different portions of a milking, however, exhibit a more or less uniform R I and a steady rise in the value of K from fore milk to strippings. But in all instances, pooling of the total yield from the animal restores the values to normal limits.

The rainy season, when lush pasture is available for cattle, appears to cause a marked rise in the limits of R I of milk, while the limits of K remain the same all through the year.

Rigorous boiling of milk causes a steady rise in the values of both R I and K.

All the data point to the fact that factors which cause a rise in the fat-free solids of milk also increase the measure of the R I. The refractive

constant, on the other hand, remains within normal limits owing to a corresponding change in the density of milk under natural conditions of variation

#### ACKNOWLEDGMENT

I wish to thank Mr B N Banerjee and Prof V Subrahmanyan for their kind interest in these studies

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## THE NATURAL OCCURRENCE OF ERGOT IN SOUTH INDIA—III

BY T S RAMAKRISHNAN

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Received January 23, 1927

(Communicated by Rao Bahadur Dr B Viswanath, CIE, DSC FRIC, FASc)

SINCE the publication of the earlier communications on the natural occurrence of ergot in South India (Thomas *et al* I, II, 1945) more hosts affected by *Claviceps* have been discovered. Descriptions of the fungi on these hosts and the results of some inoculation experiments are recorded in this communication.

1 *Cynodon plectostachyum* Pilger—This is a new grass recently introduced into Coimbatore by the Government Lecturing and Systematic Botanist for trial as a fodder. It was found to be severely infested by *Claviceps* during November 1945 to January 1946, and again in December 1946. The sphacelial form is conspicuous as white drops of sticky honey dew. A number of such drops are visible in a spike. The conidia are of two types. One kind of conidium is hyaline, oblong or reniform, measuring  $15.9 \times 6.9$  ( $11-8.5 \times 3.5-7.4$ )  $\mu$  (Fig. 1). The second type is smaller, more or less elliptical or sometimes subspherical, hyaline and measuring  $9.4 \times 7.3$  ( $7-11 \times 5.6-9.3$ )  $\mu$ . The smaller type seems to be the secondary conidia formed by the germinating bigger conidia. The sclerotia usually develop in January. They are black, slightly bent, protruding beyond the glumes and measuring  $3-5.5 \times 1.5$  mm.

This fungus closely resembles the one on *C. dactylon*. The conidial measurements and the shape and size of the sclerotia are almost identical on both the hosts and it is considered that both of them belong to the same species. A further comparison of the ergots occurring on different hosts brings out the close resemblance of the conidial stages on *Digitaria chinensis*, *D. wallichiana*, *Panicum maximum*, *Cynodon dactylon* and *C. plectostachyum*. Spore suspensions of conidia from *P. maximum* were sprayed on open flowers of *C. dactylon*. Even after 15 days there was no sign of infection. But this does not necessarily mean the fungi on these two hosts are not the same species. In the genus *Claviceps* specialisation of parasitism exists and in the same species some strains do not pass on from one host to another (Atanasoff, 1920). However without the knowledge of the stromatal and perithecial characters it is unsafe to determine this species of *Claviceps*.

2. *Vetiveria Lawsoni* Blatt and MacC.—This is another grass under experimental cultivation in the botanic Garden at Coimbatore which exhibits severe infection by *Claviceps* in December. The conidial stage appears as sticky translucent drops which finally dry up into creamy white hard round masses outside the spikelets. Sometimes the fluid spreads over the glumes and



Fig. 1.	Conidia of <i>Claviceps</i> on	<i>Cynodon pectostachyum</i> .
" 2.	" "	<i>Paspalum flavidum</i>
" 3.	Sclerotia on	<i>Paspalum flavidum</i> .
" 4.	Conidia on	<i>Cenchrus setigerus</i>
" 5.	"	<i>Vetiveria lawsoni</i>
" 6.	"	<i>Heteropogon contortus</i> .
" 7.	"	<i>Ischaemum pilosum</i>
" 8.	"	<i>Urochloa panicoides</i> .

(All drawings of conidia  $\times 500$ )

forms, on drying, white deposits over the surface of the affected and adjoining spikelets. From this white colouration the disease can be easily spotted. The conidia are hyaline, oblong with straight sides or constricted slightly in the middle. They measure  $10.2 \times 4.2$  ( $7.4-11 \times 3.7-5.5$ )  $\mu$  (Fig. 5). The diseased spikelets are soon overgrown by *Cerebella* which arrests the

formation of sclerotia though it enables one to easily locate the affected spikelets. On dissecting open some of the spikelets, not overgrown by *Cerebella*, small black sclerotia 2-3.5 × 0.75-1 mm in size can be seen between the glumes replacing the ovary. The base of the sclerotium is sometimes purple in colour.

3 *Ischaemum pilosum* Hack.—The honey dew is noticed as clear or white drops later turning brown outside the glumes. The conidia are hyaline and oblong with rounded ends. The contents are granular with the granules often grouped at the two ends. The conidia measure 11.4 × 5.0 (8.4-14.4 × 4.8-5.6)  $\mu$  (Fig. 7). Here also *Cerebella* easily overgrows the fungal tissue and sclerotial formation is thus prevented. In some of the affected spikelets with no *Cerebella* infection, small dark sclerotia measuring 3 × 0.5 mm were noticed inside the spikelets in the place of the ovary and completely enveloped by the glumes. The ergot on *Vetiveria lawsoni* and *Ischaemum pilosum* are identical and must be considered as belonging to the same species. They fall into the same group as those on *Themeda triandra*, *Ischaemum aristatum*, *Andropogon lividus* and *Cymbopogon flexuosus* (Thomas *et al* 1945). Ajrekar (1920) has recorded a *Sphacelia* on *Ischaemum pilosum* but the spores are stated to be curved. Hence the fungus recorded now is quite different.

4 *Paspalidium flavidum* A. Camus.—This is a common fodder grass found in many parts of the province. At Coimbatore it is affected by ergot in the months of November and December. The honey dew protrudes as a sticky pearly drop from the spikelet. Later it may spread over the glumes and pedicels forming white crusts. The conidia are hyaline, lunulate and measure 16 × 5 (12.8-20.8 × 4.8-6.4)  $\mu$  (Fig. 2). Sclerotia are formed. These are dark brown to black, curved 4.5 × 1 mm and projecting out from between the lemma and palea (Fig. 3).

5 *Urochloa panicoides* Beauv.—This is a good fodder grass common in all districts of the province. It is also affected by ergot at Coimbatore. The sphacelial stage develops in individual spikelets forming translucent to white drops exuding from the spikelets. Later, these harden into brown masses running over the glumes. Sometimes the whole spikelet is covered by a white deposit by means of which the affected spikelets can be easily recognised. The conidia are hyaline, fusoid to lunulate, measuring 15.4-5.1 (12.8-19.3 × 2.6-4.4)  $\mu$  (Fig. 8). The sclerotia are small, brown, globose to oblong 1.5 × 0.5-0.75 mm and occupying the position of the ovary between the lemma and palea. There is a close resemblance between the ergots on *Paspalidium flavidum*, *Urochloa panicoides*, *Urochloa reptans* and

*Brachiaria distachya* The last named host was severely infested during December 1946 at Coimbatore Thirumalachar (1945) has recorded a similar ergot on *B. distachya* from Mysore

6 *Heteropogon contortus* Beauv.—Thomas *et al* (1945) have recorded an ergot on this host having mainly triangular conidia During December 1946 another type of sphacelial infection was noticed on this host besides the one recorded before The honey-dew formation was more or less similar to the one noticed earlier but the conidia were different They were hyaline oblong, with rounded ends, very rarely tending towards reniform and measured  $15.0 \times 5.1$  ( $11-19 \times 4.8-6.4$ )  $\mu$  (Fig. 6) The honey dew soon dried into white or cream coloured masses projecting out of the spikelets

Thirumalachar (1945) has also recorded from Mysore oblong conidia of the same size in sphacelial infection of this host Thus this host appears to be susceptible to infection by two different types of *Sphacelia*

7 *Cenchrus setigerus* Vahl—This grass is common in all open places in this district A widespread epiphytotic of *Sphacelia* was noticed on this grass from the month of November, 1946 The infection involved either some of the spikelets of the panicle or all the spikelets A creamy white drop of fluid collected at the apex of the spikelet and flowed down the sides where it dried into white deposits clearly visible against the dark purple colour of the spikelet The conidia were mainly lunulate sometimes fusoid, hyaline and measured  $17.9 \times 4.8$  ( $12.8-26 \times 3.2-6.4$ )  $\mu$  (Fig. 4) The ovary and rarely the stamens also were enveloped in a compact hyphal mass From the surface of this mass large members of conidiophores were developed in a closely packed folded hymenial layer The formation of distinct sclerotia was not observed

This resembles the *Sphacelia* recorded on *Cenchrus ciliaris* (Adyantayya, 1946) and *Pennisetum hohenackeri* (Thomas *et al* 1945) Conidial suspensions of the *Sphacelia* on *C. setigerus* were sprayed on healthy blooming panicles of *C. ciliaris*, *C. setigerus*, *Brachiaria ramosa*, *Panicum maximum* and *Apluda aristata* The inoculations were carried out during a spell of rainy weather and the experimental plants were kept inside glass cages or under bell jars for three days to provide favourable conditions for infection On the ninth day a number of spikelets of *C. ciliaris*, *C. setigerus*, and *B. ramosa* were showing honey-dew formation The controls and the other hosts were free Thus the ergot passes from one host to another The conidial characters also indicate that the same species is present on *C. ciliaris*, *C. setigerus*, *B. ramosa* and *P. hohenackeri* (though inoculation experiments were not made on the last named host) The infection of the spikelets of

*C. ciliaris* is possible when inoculations are made when the anthers protrude or earlier. Six spikes were completely immersed in spore suspension for 2 minutes, three days after emergence and long before the flowers opened. In the course of ten days all the spikelets in the inoculated spikes were found infected with the honey-dew formation while the controls bloomed normally. Anthesis did not occur in the inoculated spikes.

Thurmalachar (1945) is of opinion that the ergot on *P. hohenackeri* "comes nearest to or is identical with *Claviceps microcephala*," judging from the colour of the stroma, size of ascospores and perithecia. But the conidia of this species are small and oval and measure  $7.8 \times 3.5 \mu$ , while the conidia of the fungus on *P. hohenackeri* are bigger, lunulate, and measure  $20.4 \times 5.8 \mu$  (Thomas *et al.*, 1945). Consequently it is evident that the ergot on *P. hohenackeri* is different from *C. microcephala*. Judging from the conidial characters the tentative grouping of the ergots suggested by Thomas *et al.* (1945) has to be slightly modified. In the first group in which curved and fusoid conidia are observed the two subdivisions may be modified as follows —

Nature of conidia	Host plants
(1) Conidia of various shapes	
(a) Conidia reniform	<i>Cynodon dactylon</i> <i>C. plectostachyum</i> <i>Digitaria chinensis</i> <i>D. wallichiana</i> <i>Panicum maximum</i>
(b) Conidia lunulate or fusoid	<i>Urochloa reptans</i> <i>U. panicoides</i> <i>Apluda aristata</i> <i>Cenchrus ciliaris</i> <i>C. setigerus</i> <i>Pennisetum hohenackeri</i> <i>Brachiaria ramosa</i> <i>B. distachya</i> <i>Paspalum flavidum</i>

8 *Sorghum* spp. — The occurrence of *Sphacelia sorghi* on many varieties of cultivated grain sorghums (*S. vulgare*, *S. aurra*, *S. Roxburghiana*, etc.) have been recorded from various parts of India, Burma and Africa. During this year the sugary disease was observed on a number of wild or exotic sorghums also at the Millets Breeding Station, Coimbatore, during December. The species that were involved are *S. halepense* Pers., *S. arundinaceum* Stapf,

*S. verticilliflorum* Stapf, *S. nitens* (B & P) Snow, *S. cafferorum* Beauv., and *S. membranaceum* Chiov. In all these pearly drops were seen exuding from the spikelets. These later dried into white deposits sometimes connecting together the contacting spikelets. The ground underneath the affected plants was bespattered with white spots. The conidia in all cases were alike being oblong with rounded ends and slightly constricted in the middle measuring on an average  $16 \times 7 \mu$  ( $12.19 \times 5.8$ ). The size, shape of conidia, and the symptoms of infection are similar on all the hosts and agree with those of *Sphacelia sorghi* McRae. Sclerotial formation was not in evidence on any of the hosts.

#### SUMMARY

Six new hosts of *Claviceps* have been recorded and the fungal characters on these hosts are described. These fungi fall into one or the other of the groups previously recorded by Thomas *et al.* (1945) for the ergots occurring in South India. A slight modification of the grouping adopted by Thomas *et al.* (1945-1) in classifying the ergots by the conidial characters, has been made. Wild and exotic species of sorghum were infected by *Sphacelia sorghi*.

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# PHYTOPHTHORA PALMIVORA BUTLER CAUSING A SEEDLING BLIGHT OF HIBISCUS ESCULENTUS L \*

BY M S BALAKRISHNAN, M Sc

Received April 18, 1947

(Communicated by Rao Bahadur Dr B V Nath, F A S C I E, D S C F R I C)

WHILE raising seedlings of *Hibiscus esculentus* L. for inoculation experiments with a species of *Pythium* recently isolated from that host, it was noticed that there was severe collar rot and seedling blight in many of the pots. Most seedlings were attacked while still in the cotyledonary or four-leaved stage and infected plants soon wilted, fell over due to rotting at the collar, and died (Pl VII Fig 1). Infection was very severe during rainy spells followed by cloudy days resulting in losses ranging from 85 to 90 per cent. During one week in particular when there was very heavy rain for three days followed by overcast weather all the 180 seedlings in 25 pots were killed. Examination of blighted seedlings showed extensive rotting of the collar region often followed by stem and root rot (Pl VII, Fig 2).

The causal organism was isolated by using bits of surface sterilised tissue taken from the margin of the rotted portions and small bits of rotted stems and roots. Out of a total of 20 isolations, 17 yielded pure growths of a species of *Phytophthora*. In the remaining three instances *Rhizoctonia solani* was present along with *Phytophthora*. However, as *Phytophthora* was present in all cases and as 17 out of 20 isolations yielded pure growths of this fungus, it was assumed that this was the causal agent and that *R. solani* was only a secondary parasite. Inoculations carried out later with pure cultures confirmed this conclusion.

Isolates of this fungus grew well on most culture media, growth being especially luxuriant on oatmeal, frenchbean and carrot agars. Of these three, oatmeal was found to be the best and was used for maintenance of pure cultures. The organism develops copious coarse aerial mycelium on the culture media mentioned above and both sporangia and chlamydospores are produced in abundance in cultures over 22 days old.

The sporangia (Pl VI, B C) were sub-spherical or limoniform, papillate, terminal or intercalary and measured on an average  $35 \times 25 \mu$  (the range being  $20-40 \times 18-35 \mu$ ). The zoospores were reniform and laterally

\* Contribution from the Mycology Section, Agricultural Research Institute, Coimbatore.

biciliate measuring 8 to 12  $\mu$  by 6 to 8  $\mu$  while swimming (Pl VI, Ia, b, c, d) and 8 to 10  $\mu$  when encysted (Pl VI, J, a) After encystment they germinated by producing one to three germ tubes (Pl VI, J, b, c, d, e)

The chlamydospores (Pl VI, D, E, F, G) were spherical or sub-spherical, terminal or intercalary and ranged from 20 to 35  $\mu$  in diameter (average 28.5  $\mu$ )

The measurements of sporangia and chlamydospores given above fall within the range given by other workers for *Phytophthora palmivora*, a species which occurs commonly in South India As the present isolate did not form oospores in pure culture even after three months, paired cultures were made with known plus and minus strains (Thomas *et al.*, 1947) of *P. palmivora* available in the stock culture collection at the Mycology Section, Coimbatore, in an attempt to induce oospore formation The results of these trials are shown in the table below

Isolate with which the <i>H. esculentus</i> Phytophthora was grown in paired culture				Result	
1	<i>Phytophthora palmivora</i>	(Plus strain)	isolated from tree a te Au S Kanara	Oospores formed in	4 days
2	do	(Plus strain)	isolated from tomato Coimbatore	do	36 hrs *
3	do	(Plus strain)	isolated from <i>Calceolarius</i> S Kanara	do	3 days
4	do	(Plus strain)	isolated from <i>Clerodendron infortunatum</i> S Kanara	do	3 days
5	do	(Plus strain)	supplied by Dr Uppal Bombay	do	3 days
6	do	(Plus strain)	isolated from <i>Cyphomandra betula</i> Berliar	do	36 hrs *
7	do	(Minus strain)	supplied by Dr Uppal Bombay	No oospores formed	
8	do	(Minus strain)	isolated from <i>Spontia mangifera</i> S Kanara	do	
9	do	(Minus strain)	isolated from <i>Carica papaya</i> , Coimbatore	do	

\* The rapidity with which oospores are formed in these two instances is due to the freshness of the isolates (*cf* Thomas *et al.* 1947)

These results show that the *H. esculentus* isolate is a minus strain of *P. palmivora*

The oogonium is spherical or sub-spherical with a fairly slender stalk encompassed by the persistent amphigynous antheridium (Pl VI, K, L, M, N, O, P) While young, the oogonia had hyaline walls and granular contents which became brownish after fertilization and the differentiation of the oospore The oospores were usually spherical, thick-walled and not quite filling the oogonium When ripe, the walls of the oogonia and

oospores were golden brown to dark brown in colour. Oogonia ranged from 20 to 40  $\mu$  (av. 30.5  $\mu$ ) in diameter and oospores from 18 to 30  $\mu$  (av. 23.5  $\mu$ ). These sizes are well within the range given by previous workers for oospores and oogonia formed in paired cultures of *P. palmivora* (Ashby, 1929; Davidson, 1934; Gadd, 1924; Lester-Smith, 1927; Marudharajan, 1941; Narasimhan, 1930; Thomas *et al.*, 1947; Thompson, 1924 and Venkatarayan, 1932).

Inoculations were carried out with this fungus on *H. esculentus* seedlings using the terminal bud inoculation technique described by Wiant and Tucker (1940) for *Phytophthora capsici* Leonian. A severe die-back and rotting of the younger portions resulted, the infected plants being killed in 10 to 15 days.

In addition, soil inoculations were also carried out. The procedure adopted was as follows. Good viable seeds of *H. esculentus* were treated with an organo-mercury compound (usually Agrosan—GN) and then sown in sterilized soil in glazed pots. Sterile distilled water was used in subsequent watering to obviate the possibility of water-borne contamination. When the seedlings were about 10 to 12 cm tall, a hole was made in the soil in the centre of the pot, roughly 8 cm away from the seedlings, care being taken to see that the roots of the young plants were not injured and bits of agar cultures of the fungus introduced, the hole was then covered up and the pot kept covered by a sterilized bell-jar. Suitable controls were also set up simultaneously. Infection was observed in the inoculated pots 7 to 10 days after inoculation with typical symptoms of collar-rot, wilting and tendency to fall over. Infected plants were killed in 13 to 17 days. All controls remained healthy and unaffected. The pathogen was successfully reisolated from diseased seedlings.

Young and old fruits of *H. esculentus* were also inoculated with small bits of agar cultures after surface sterilization and kept covered with a sterilized bell-jar. The fruits took infection readily and rotted within five days. In all cases the controls remained healthy and unaffected.

A survey of literature shows that so far only one member of the Pythiaceae—*Pythium debaryanum* Hesse—has been recorded on *Hibiscus esculentus* L. (Ramos, 1926). No species of *Phytophthora* has till now been reported to attack this plant though *P. parasitica* Dastur and *P. palmivora* have been recorded on *Hibiscus sabdariffa* (Tucker, 1933; McRae, 1932; Thompson, 1933), *H. sabdariffa* var. *altissima* (Tucker, 1933; Hector, 1931; Kar and Saha, 1943), and *H. manihot* (Tasugi and Ikeda, 1939) and

*P. palmivora* Butler Causing Seedling Blight of *H. esculentus* L 145

*H. cannabinus* (Muller and Van Eek 1939) This appears to be the first record of *P. palmivora* on *Hibiscus esculentus*

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# SUMMARY

*Phytophthora palmivora* Butler was isolated from blighted seedlings of *Hibiscus esculentus* L Inoculation experiments proved its pathogenicity A study of its cultural and sexual behaviour showed that it was a minus strain not forming oospores in pure culture and forming them only when grown in paired cultures with complementary (plus) strains of the same species

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## EXPLANATION OF PLATES

## PLATE VI

A	Vegetative hypha
B and C	Terminal and intercalary sporangia
D to G	Terminal and intercalary chlamydo-spores
H	Liberat on of zoospores
I	Free swimming zoospores
J	Encysted (a) and germinating (b-e) zoospores
K to P	Oogonia and oospores of the <i>Hibiscus esculentus</i> Phytophthora with other isolates of <i>P. palmivora</i>
K	<i>H. esculentus</i> × Tomato
L	do × <i>Cyphomandra betacea</i>
M	do × <i>Areca catechu</i> (S Kanara)
N	do × do (Dr Uppals isolate)
O	do × <i>Clerodendron unfortunatum</i>
P	do × <i>Colocasia antiquorum</i>

Note—All figures were drawn with the aid of an Abbe camera lucida at table level  
The magnification in the case of figures A-H is  $\times 540$  and figures I—P  $\times 765$

## PLATE VII

Photographs of diseased *H. esculentus* seedlings

Fig. 1 Diseased (A) and healthy (B) potted seedlings

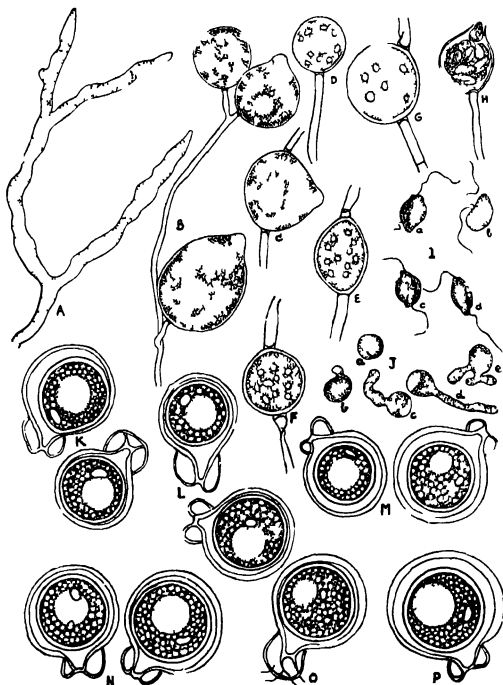
Fig. 2 Seedlings showing different stages of infection (A) Start of infection note lesion at the collar region (B) and (C) more advanced about half the stem is rotted in (C), (D) plant completely rotted and dead



Fig 1



Fig 2



## STUDIES IN THE GENUS PHYTOPHTHORA

### I. Oospore Formation and Taxonomy of *Phytophthora palmivora* Butler

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(Communicated by Dr. B. Viswanath, C.I.E., D.Sc., F.R.I.C.)

THE development of the sexual bodies in *Phytophthora palmivora*, Butl. has been investigated by a number of workers from time to time. Aided by these and other physiological reactions the status of this species has been revised every now and then and modified to take in more than one species created by later workers in its fold. The arguments put forward by the revisionists have been varied.

Coleman (1907) was the first to report on oospore formation in cultures of *P. omnivora* var. *arecae* Colem. [(later named *P. arecae* (Coleman) Pethy]. Rosenbaum (1917) obtained oospores of *P. arecae* in cultures received from Coleman. But other workers have not been able to obtain these sexual bodies in single strain cultures. Narasimhan (1930) has put forward the explanation that the cultures sent to Rosenbaum were probably not a single strain but a mixed one and that this might be responsible for the formation of oospores. The same explanation is applicable for the development of oospores in Coleman's cultures also. McRae (1917) has described the production of oospores in single strain cultures of *P. meadit* McRae. But this ability is shortlived and other workers to whom cultures of this fungus had been supplied found that oospores did not develop in the cultures. The isolate now studied also does not produce oospores in single strain cultures. Ashby (1922) noted that in paired cultures of *P. palmivora* and *P. faberi* Maub. (coconut and cacao strains) oospores developed. Later (1929) he continued the studies on the development of sexual bodies in paired cultures of *Phytophthora* and has recorded their formation in paired cultures of isolates from coconut and cotton; citrus and coconut; rubber and cacao; and coconut (India) and coconut (Jamaica). As a result of these studies and with isolates from other sources he recognised that the isolates he had, could be placed into two groups, the members of one group forming oospores when mixed with members of the other. Adopting Gadd's (1925) group nomenclature, he arranged the isolates into the 'cacao' and 'rubber'

groups. In the 'cacao' group were isolates from cacao, coconut (India), papaya, *Vanda* and *Cattleya* and in the 'rubber' group isolates from coconut (Jamaica and Philippines), Citrus, *Hevea*, *Dendrobium* and *Odontodenia*. Lester-Smith (1927) grew three isolates of *P. faberi* Maubl. in paired cultures and obtained oospores. In combination with *P. parasitica* Dast also *P. faberi* formed oospores. Paired cultures of *P. parasitica* strains and *P. nicotianæ* Br de Haan also produced oospores. Gadd (1925) made a comparative study of the strains of *Phytophthora* isolated from cacao, papaya, *Hevea*, *Dendrobium*, *Odontodenia* and breadfruit in Ceylon. He found that these strains produced oospores in paired cultures, the isolates from cacao and papaya behaved as 'plus' strains and others as 'minus' strains. His later studies (1927) have confirmed his earlier conclusions but he found that the isolates from areca in Ceylon did not form oospores with isolates of *P. faberi* strains. Thompson (1929) obtained eight isolates of *Phytophthora* from *Hevea brasiliensis* which he classified into *P. palmivora*, *P. meadii*, and *P. heveæ* Thompson. These formed oospores in paired cultures with other strains (coconut and roselle) of *P. palmivora* and *P. parasitica*. Narasimhan (1930) studied oospore formation in paired cultures of isolates from *Areca*, *Santalum album* L., *Loranthus longiflorus* Desv., *Jatropha curcas* L., *Bryophyllum calycinum* Salisb., *Artocarpus integrifolia* L., *Colocasia antiquorum* Schott and *Ficus hispida* L. He found that in paired cultures the isolates from *Loranthus* and *Areca* developed oospores with the isolates from *Santalum* and *Jatropha*. Leonian (1931) studied the behaviour of 85 cultures of *Phytophthora*. He found that 48 of them were heterothallic equally divided into males and females while the remainder were classified into inconstant forms exhibiting heterothallism, and neutral behaviour. The cultures he tested included *P. palmivora*, *P. faberi*, *P. parasitica*, *P. terrestris* Sherb., *P. manoana* Sid. and *P. nicotianæ*. Venkatarayan (1932) was able to obtain oospores in paired culture of two isolates of *P. arecæ* and *P. palmivora*. Uppal and Desai (1939) obtained oospores in paired cultures of two isolates of *P. arecæ* from Bombay Province. Marudarajan (1941) investigated the formation of oospores in six isolates from *Areca*, *Hevea*, coconut, palmyra and Cacao and agreed with Gadd in the existence of two groups. He continued his investigations with two isolates from *Clerodendron infortunatum* L. and *Spondias mangifera* Willd., each belonging to one of the above groups.

#### MATERIALS AND METHODS

The availability in the stock cultures of the Government Mycologist, Coimbatore, of a large number of isolates of this genus and of *P. palmivora* as it is now understood was taken advantage of to study their sexual behaviour.

under controlled cultural conditions. The list of isolates used in these studies with the accepted identifications as far as they were known at the commencement of these studies is given in Table I

TABLE I

*List of the isolates of Phytophthora used in the study of paired cultures*

S. No.	Host	Part affected	Pathogen	Locality	Source from which the isolate was obtained
1	<i>Agave sagittatus</i> , Dr and Fr.	Leaf	<i>Phytophthora farwellii</i> Dast	Coimbatore	Local isolation from Madras Province
2-6	<i>Areca catechu</i> L. (Four isolates) (One isolate)	Fruit	<i>P. arecae</i> (Colim) Pethy.	South Kanara	do
	do	do	do	Mysore	Mr M J Narasimhan, Bangalore
	do	do	<i>P. arecae</i> (Strain Tyagli)	North Kanara	Dr B N Uppal, Poona
	do	do	<i>P. arecae</i> (Strain Nilkani)	Bombay	do
7	<i>Artocarpus lacucha</i> L.	do	<i>P. palmivora</i> Butl.	South Kanara	Local isolation from Madras Province
8	<i>A. integrifolia</i> L.	do	<i>P. arecae</i> (Colim) Pethy	do	do
9	<i>Borassus flabellipes</i> L.	Bud	<i>P. palmivora</i> Butl.	Malabar	do
10	<i>Citrus nobilis</i> Lou, (II)	Leaf and fruit	do	do	do
11	<i>Citrus sinensis</i> Osbeck (II)	Base of stem	<i>P. palmivora</i> Butl.	Kistna	Local isolation from Madras Province
12	<i>Clerodendron infortunatum</i> L.	Leaf	<i>P. sp.</i>	South Kanara	do
13	<i>Cocos nucifera</i> L.	Bud	<i>P. palmivora</i> Butl.	Malabar	do
14	<i>Colocasia antiquorum</i> Schott.	Leaf	<i>P. sp.</i>	South Kanara	do
15	<i>Hevea brasiliensis</i> M. Agg.	Leaf and fruit	<i>P. meadii</i> McRae	Colim State	do
16	<i>Lycopersicon esculentum</i> Mill.	Fruit	<i>P. arecae</i> (Colim) Pethy.	Coimbatore	do
17	<i>Nicotiana tabacum</i> L.	Stem	<i>P. parasitica</i> var. <i>nicotianae</i>	Salem	do
18	<i>Piper betle</i> L. (3 isolates)	do	<i>P. palmivora</i> Butl.	Chingleput and Tanjore	do
19	<i>Spondias mangifera</i> Willd.	Fruit	do	South Kanara	do
20	<i>Theobroma cacao</i> L.	do	<i>P. faberi</i> Maubl.	Ceylon	Government Mycologist, Ceylon
21	<i>Jatropha curcas</i> L.	do	<i>P. sp.</i>	South Kanara	Local isolation from Madras Province

All the isolates were pure strains and non-oospore forming at the time when the study was commenced. Some of them have been reported to have produced oospores in single strain culture but at the time of the studies no

oospores could be detected in any of the cultures. The isolates from agave, breadfruit and *Hevea* come under this class. Paired cultures were grown in petri dishes or agar slants. In petri-dishes, quadrants were marked on the dishes by cutting out furrows 2-3 mm wide in the media through the centre of the dish at right angles. The two strains used in paired cultures were inoculated on adjacent quadrants so that oospore formation if any could be detected easily in the clear furrows where the two growths meet when examining the undersurface of the dish under the low power of the microscope. On agar slants in tubes the two strains were placed side by side on one edge of the slant half way down its length so that the periodic examination of the tube under the low power of the microscope was facilitated. Except when otherwise mentioned, the cultures were grown on oat agar media at laboratory temperature.

#### RESULTS OF EXPERIMENTS

*Experiment I*—At the outset the two isolates from *Areca* which were obtained from Dr Uppal were grown together, oospore development was observed on the fifth day in the furrows between adjacent quadrants. In the course of ten days numerous oospores had developed in the zones of both the strains besides those formed in the furrow. Dark lines or zones representing the areas of oospore formation described by Narasimhan (1930) were, however, absent.

*Experiment II*—The next step was to find out the sexual behaviour of the different isolates from *Areca* available at Coimbatore. Each of the five isolates, four from South Kanara and one from Mysore, was grown together with each of the two Bombay strains. It was found that all these five isolates formed oospores with the Tyagli strain but not with the Nilekani strain. This explains why Marudarajan (1941) failed to get oospores in mixed cultures of the areca strains from this province. Obviously all of them happened to belong to the same group and further studies revealed that these isolates corresponded with the Nilekani strain from Bombay.

*Experiment III*—In a third series of experiments a large number of the isolates of *Phytophthora* available at Coimbatore and originally isolated from a variety of hosts were grown in paired cultures with the two Bombay strains of *P. areca* with the following results (Table II).

It is clear from Table II that all the isolates used in this experiment fall into two distinct groups, one forming oospores with the Tyagli strain and the other with the Nilekani strain.

*Experiment IV*—Paired cultures were then made of various permutations and combinations of all isolates available at Coimbatore other than

TABLE II

Results of paired culture studies made with two Bombay isolates of *P. arecae*

Isolate	Result of pairing with	
	Tyagii strain	Nilekani strain
Agave ..	No oospores	Oospores formed
Breadfruit ..	do	do
Citrus II ..	do	do
Coconut ..	do	do
Hevea ..	do	do
Jatropha ..	do	do
Palmyra ..	do	do
Cocoa ..	do	do
Spondias ..	do	do
Areca ..	Oospores formed	No oospores
Betel vine (3 isolates) ..	do	do
Citrus I ..	do	do
Clerodendron ..	do	do
Jak ..	do	do
Tomato ..	do	do

the two Bombay isolates of *P. arecae* supplied by Dr Uppal and observations on the formation of oospores were recorded

The results are given below—

TABLE III

Results of paired culture studies among original collections of *Phytophthora* available at Coimbatore

Isolates	Areca	Betel Vine	Cit. I	Clerodendron	Jak	Tomato	Agave	Breadfruit	Cocoa	Cit. II	Coconut	Citron	Hevea	Jatropha	Spondias	Palmyra	Tobacco
Areca ..	0	0	0	0	0	0	x	..	x	x	x	0	x	x	x	x	x
Betel vine ..	0	0	0	0	0	0	..	..	..	x	x	..	x	x	x	..	..
Citrus I ..	0	0	0	..	..	0	x	..	x	x	x	0	x	x	x	x	..
Clerodendron ..	0	0	..	0	0	0	x	..	x	x	x	..	x	x	x	x	..
Jak ..	0	0	..	0	0	0	..	..	..	x	x	..	..	x	x	x	..
Tomato ..	0	0	0	0	0	0	x	x	..	x	x	..	..	..	..	x	x
Agave ..	x	..	..	..	x	x	0	0	0	..	0	..	0	0	0	0	..
Breadfruit ..	x	x	x	x	x	x	0	0	..	..	0	0	x	..	..	..	..
Cocoa ..	x	x	x	x	x	x	0	..	0	0	0	..	0	0	0	0	..
Citrus II ..	x	x	x	x	x	x	..	0	0	0	0	..	0	0	0	0	0
Coconut ..	x	x	x	x	x	x	..	0	0	0	0	..	0	0	0	0	0
Citron ..	0	..	..	0	..	..	..	..	..	..	..	..	..	..	x	..	..
Hevea ..	x	x	x	x	x	x	0	..	0	0	0	..	0	0	0	0	..
Jatropha ..	x	x	x	x	x	x	0	..	0	0	0	..	0	0	0	0	..
Palmyra ..	x	x	x	x	x	x	0	..	0	0	0	..	0	0	0	0	0
Tobacco ..	x	..	..	..	x	x	..	0	..	..	0	..	0	0	0	0	0
Spondias ..	x	x	x	x	x	x	0	0	..	..	0	x	0	0	0	0	..

*Remarks*—The isolate from breadfruit died soon after the commencement of this experiment, it could not be utilised for further study of paired cultures

0 = No Oospores    × = Oospores formed    = Combinations not tried

The behaviour of these isolates has been consistent and in conformity with the results obtained in previous experiments. The isolates fall into two sexually distinct types. The members of one group invariably form oospores in paired cultures with members of the other group but not amongst themselves.

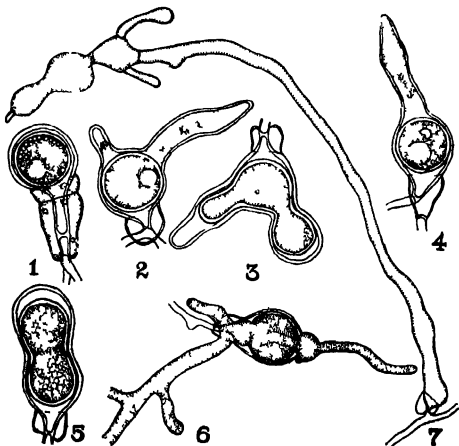
#### MORPHOLOGY OF THE SEXUAL ORGANS

The oogonia are spherical, thick walled, light yellow to yellowish brown in colour, persistent and always with one amphigynous antheridium at the base. Sometimes double antheridia may be seen one above the other (Text-Fig 1). The antheridia and oogonia always developed on different hyphae. No instance of the two organs developing on the same hypha was seen. The oogonial wall exhibits variations. In some combinations all the oogonia were smooth, but in others like *Citrus I* × *Jatropha*, *Citrus I* × *Citrus II*, *Citrus I* × *palmyra* and *Citrus I* × *coconut* some of the oogonia had thicker walls which were not as clear cut as in others but possessed a rough outline due to uneven thickening of the outer surface (Figs O, P, Q). This feature has been noted by Gadd (1924) in some of the paired cultures of isolates from Ceylon. Tompkins and Tucker (1937) observed a thick brown crystalline encrustation on the wall of the oogonium produced in paired cultures of *P. capsici* Leon. Consequently the exact limits of the oospore and oogonia could not be determined while taking measurements. With the addition of concentrated solution of potassium hydroxide the encrustation disappeared and the walls of the oogonia and oospores became clear. The thickening of the oogonial wall noticed in some of the cultures under study however did not react in a similar manner when potassium hydroxide solution was added.

The oospores are spherical, thickwalled and light yellow to yellowish-brown or reddish-brown in colour. They may either completely fill the oogonial cavity or there may be some space between the two walls. In all paired cultures varying proportions of the two kinds can be observed.

In some of the cultures (e.g., *Citrus I* × *Jatropha*) some of the later formed oogonia and oospores were peculiar. The oogonium was elongated, irregularly swollen and sometimes developing one or more branches (Text-Figs 2-5, 7). Usually only one oospore was found in such oogonia but this occupied only a portion of the oogonium while the rest of the cavity

was either empty or filled with vacuolate hyaline or yellowish protoplasmic contents. Oospores also exhibited lobulation in some cases. Some of the oogonia were very much elongated and branched and usually these did not contain oospores. They resembled empty swollen elongated and branched hyphae with scanty or disintegrated contents. The presence of the amphigynous empty antheridium at the base distinguished these structures from vegetative hyphae and showed them as malformed oogonia. Leonian (1931) has observed similar structures in a culture of *P. palmivora*. But he describes them as germinating oospores and the round bodies inside some of them as secondary oospores. But the writers do not agree with this. These structures represent abnormal proliferating oogonia. The



TEXT FIGS 1-6—Sexual bodies from Citrus I X *Jatropha*. 1 Double antheridia 2 5 Abnormal oogonia with differently shaped oospores 6 Germinating oospore 7 An abnormal oogonium showing peculiar growth and branching ( $\times 680$ )

germination of the oospore can be easily distinguished by the presence of the germ tube which bursts through the wall of the oospore and later through the wall of the oogonium and grows out (Text-Fig 6). But in most of the cases this was not evident and the oogonium alone had elongated and developed branches. When the oospore germinates the oogonium does not elongate. The sketches given by Leonian (1931) also do not bear out his statement. They can be regarded only as abnormal oogonia. Those oogonia which have not been fertilised probably resume vegetative activity and develop into branched structures of limited growth.

In tubes and petri-dishes the sexual bodies develop on the medium or are submerged, they are also formed on the sides of the glass in tubes and in the clear space between the quadrants in petri-dishes. The first formed oospores are usually at the junction of the growths from the two isolates.

TABLE IV

*Measurements of oogonia and oospores as recorded by various workers*

Author	Species or pair of isolates	Oogonia		Oospores	
		Range $\mu$	Mean $\mu$	Range $\mu$	Mean $\mu$
Rosenbaum	<i>P. arecae</i> ..			23-44	33.4
Coleman	do			23-36	
Ashby	Cacao+Coconut 3 ..	22-35	28.6	17.8-27.8	23.1
	Cacao+Cotton boll ..	do	29.0	17.8-26.6	23.1
	Cacao+ <i>P. parasitica</i> (castor) ..	25-33	28.8	20.0-25.3	23.6
	Coconut (Jamaica)+Coconut (India) ..			26-39	31.8
	<i>Hevea</i> +Cacao (Hevea Zone) ..			23-33	27.6
	(Cacao Zone) ..			20-30	25.0
	<i>P. meadus</i> + <i>P. arecae</i> ..	24-34	28.5	20-28	24.0
	<i>P. meadus</i> (Malaya)+ <i>P. arecae</i> ..	24-35	29	23-28	25.3
McRae	<i>P. meadus</i> ..	24-35	33	16-32.8	25.0
Gadd	Cacao+ <i>Odontolaena</i> ..			19-25	23.1
				21-28	23.8
	Cacao+rubber ..			20-28	23.7
	Cacao+Breadfruit ..			23-27	24.0
	Papaw fruit+ <i>Odontolaena</i> ..			17-28	23.4
Narasimhan	<i>Areca</i> + <i>Santalum</i> ..			30-31	
	<i>Areca</i> + <i>Jatropha</i> ..			26-27	
	<i>Santalum</i> + <i>Loranthus</i> ..			30-31	
	<i>Loranthus</i> + <i>Jatropha</i> ..			29-30	
Venkatarayan	<i>Aleurites</i> + <i>areca</i> (fruit) ..		27.8		23.04
	do + do (top rot) ..		25.8		23.3
	<i>Areca</i> (top rot)+ <i>Santalum</i> ..		25.9		25.5
	<i>Areca</i> (fruit) + do ..		22.6		23.1
Marudarejan	<i>P. arecae</i> + <i>P. meadus</i> ..	28-43	35.7	24.5-33.5	31.5
	do + <i>Palmyra</i> ..	28-40	33.6	do	29.5
	do + <i>Coconut</i> ..	28-42	36.0	do	30.8
	do + <i>Citrus</i> ..	24.5-33.8	29.4	22.8-31.5	27.3
	do + <i>Cacao</i> ..	18.3-33.3	28.0	17.5-29.8	24.5

Later they may be observed in other portions also. In some combinations oospores are formed in plenty while in others they are few. This difference in the intensity of formation of sexual bodies may be due to the fact that the isolates were originally brought into culture at different periods and consequently varied in the number of generations they had passed through in subcultures on agar media.

The size of the oogonium and the oospore exhibited wide variations. The measurements of the oogonia and oospores obtained by previous workers are given in Table IV.

These measurements were compared with those of the sexual bodies produced in the paired cultures under study. One hundred measurements were made in each case. The sexual bodies were taken from paired cultures within ten to fifteen days after inoculation (Table V).

TABLE V  
Measurements of sexual bodies obtained from different combinations

Serial No.	Isolates grown in paired cultures	Diameter			
		Oogonia		Oospores	
		Range in $\mu$	Mean $\mu$	Range in $\mu$	Mean $\mu$
1	<i>Arca</i> (Nilakani + Palmy)	23-41	30.0	18.6-30.0	24.4
2	<i>Arca</i> (Nilakani) + <i>Jatropha</i>	21.7-40.3	28.6	14.1-31.0	21.8
3	<i>Arca</i> (Nilakani) + <i>Spondias</i>	14.0-31.3	23.9	10.5-28.0	17.5
4	Jak + Palmyra	20.2-27.0	25.1	15.5-21.7	18.5
5	Jak + <i>Jatropha</i>	21.7-14.1	27.1	15.5-26.9	20.9
6	do + <i>Citrus</i> II	21.7-34.1	26.5	14.0-27.0	23.0
7	do + <i>Hevea</i>	20.2-34.1	27.2	15.5-27.0	21.8
8	do + <i>Agave</i>	21.7-31.0	25.8	15.5-24.8	19.9
9	do + <i>Spondias</i>	23.1-34.0	28.4	18.6-27.0	22.5
10	do + <i>Arca</i> (Tyngali)	24.1-34.4	29.8	18.6-31.0	23.3
11	<i>Citrus</i> I + <i>Spondias</i>	21.7-14.1	27.1	15.5-27.0	21.6
12	do + <i>Citrus</i> II	24.8-37.2	30.6	18.6-31.0	24.7
13	do + Palmyra	21.7-34.1	28.1	17.1-28.4	21.6
14	do + <i>Jatropha</i>	24.8-37.2	29.1	18.6-31.0	23.0
15	<i>Clerodendron</i> + <i>Hevea</i>	21.7-32.6	27.5	17.1-24.8	21.1
16	<i>Clerodendron</i> + <i>Spondias</i>	23.3-34.1	29.4	18.6-31.0	24.0
17	Betal vine I + <i>Spondias</i>	21.7-32.6	27.5	18.6-27.0	21.7
18	Betal vine II + <i>Spondias</i>	24.8-37.2	30.0	18.6-31.0	23.8
19	Betal vine + <i>Arca</i> (Tyngali)	24.8-36.5	28.4	15.5-31.0	21.9
20	<i>Citrus</i> I + Tyngali	21.7-30.0	27.5	15.5-24.8	21.0
21	<i>Clerodendron</i> + <i>Arca</i> (Tyngali)	24.8-35.7	30.5	17.1-27.0	22.4
22	<i>Arca</i> (Kanara) + <i>Spondias</i>	21.7-34.1	28.4	18.6-27.0	22.4
23	<i>Calocarpus</i> + <i>Spondias</i>	21.7-34.1	27.6	15.5-24.8	21.9
24	Tomato + Tobacco	20.2-27.0	24.1	15.5-21.7	19.0
25	Tomato + <i>Jatropha</i>	21.7-31.0	25.1	18.6-27.0	21.9
26	Tomato + <i>Citrus</i> II	21.7-34.1	29.1	18.6-27.0	24.8
27	Tomato + Coconut	23.3-34.1	27.7	20.2-29.6	23.4
28	Breadfruit (alone) (suspected to be mixed)	20.0-28.5	25.0	16.5-24.5	20.6
29	Tomato + <i>Hevea</i>	24.8-34.1	28.1	20.2-29.6	24.1

The mean diameter of the oospores varies from 17.5 to 24.4  $\mu$  and lies within the range obtained by other workers. The wide variation in size of the sexual bodies observed in these studies and by other workers goes to show that this character is highly variable and plastic and that no reliance can be placed on this for taxonomic purposes. However the ability to form the oospores in paired culture brings out the specific relationship of the complementary isolates.

#### SOME PHYSIOLOGICAL STUDIES

*The influence of medium on oospore formation*—It has been stated by previous workers that certain media favoured the formation of oospores in mixed cultures while others did not. This factor differs with isolates. Thus Tucker (1931) found that certain isolates of *P. parasitica* produced large numbers of oospores in lima bean and oat-meal agars and few or none on cornmeal agar, while still others developed more oospores on cornmeal agar and a smaller number on oat-meal agar. The investigations on paired cultures recorded here were carried out on oat agar which was found to be quite satisfactory. Leonian (1931) also found that oat agar was the most suitable. Two complementary strains, viz, *Citrus I* and *Citrus II* were grown on oat, frenchbean and maize agars. The reaction of the media was adjusted to pH 5.6 in each case. The growth of the fungi was very luxuriant on oat and frenchbean agars and less profuse on maize agar. Oospores were formed in all cases but were more numerous in french-bean and oat agars than in maize agar. Tucker's observations only show the possibility that different races have preference to particular media for growth and reproduction.

Liquid oat-extract was prepared by boiling 50 gm. of powdered oat grains in a litre of water for one hour and then filtered through cottonwool. After filtering, the extract was autoclaved for 20 minutes at 15 lbs. pressure. In this medium two strains were grown for 15 days after which the medium was filtered through Chamberland filters under aseptic conditions. Five and ten cubic centimetres of the filtrates were mixed with 10 c.c. of melted oat agar medium which was then poured into plates. After the agar had set, the plates were inoculated with the complementary strain. Even after 30 days' growth oospores were not formed. This indicates that a strain does not secrete any extra-cellular substance into the medium to stimulate oospore formation in its complementary strain. Further work on these lines is in progress.

*Temperature and oospore formation*—Ashby (1929) has recorded that if paired cultures are maintained at 23° C. (or 20°–25° C.) prompt development

of oospores takes place. Other workers also have experienced that exposure to lower temperatures or maintenance of cultures in ice-chests is conducive to oospore formation. Marudarajan (1941) observed oospore formation to be good at 20° C. In the course of the present investigation it was observed that oospores did not develop in the paired cultures started in the months of March, April and May when the laboratory temperatures varied between 28° C and 31° C. But in July and August and from October to January the paired cultures readily produced oospores when the laboratory temperature was below 26° C. Paired cultures kept inside a controlled temperature cabinet in which the temperature varied from 8°–10° C failed to develop oospores. Very low temperatures evidently do not favour the development of oospores in this tropical species.

*Age of isolates and oospore formation*—In fungi, it is common experience that the intensity of sporulation gradually diminishes as the isolate is kept on for a large number of generations on agar media and may even disappear eventually. This behaviour is often seen in *Phytophthora* especially with regard to oospore formation. Paired cultures of fresh isolates of complementary strains produce oospores quite readily and in large numbers in 3–8 days depending on the distance separating the inocula of the two strains. But after several generations of sub cultures the capacity to form oospores decreases in some strains until it is finally lost. For instance the Nilekani strain on *Areca* obtained from Bombay used to form large numbers of oospores with its complementary strains as mentioned earlier. But at the time of writing, i.e., two years after its arrival, it does not form oospores with the isolates with which it was forming oospores before. It has become neutral.

The isolate from *Spondias* is another good example of the waning of the capacity to form oospores with ageing. A fresh isolate formed oospores with all the complementary isolates in four days. Another which had been isolated two years ago produced sexual bodies in combination with the same complementary strain. But the development was incomplete. Antheridia and oogonia were formed but mature oospores did not develop. The oogonia had grown through the antheridia and assumed the normal size and shape after emergence but later the contents disintegrated. Six months later, even this phenomenon did not occur in the combinations. Thus there has been a gradual decline of the sexual capacity of the isolate. This phenomenon is attributable either to the senescence of the isolate through successive subculturing on media for a long period or to formation of indistinguishable dissociants which were neutral or had lost their sexuality. These dissociants

have possibly been carried over in the transfers and thus the change might have occurred. Further experiments are necessary to decide the correctness of this view.

#### DISCUSSION

The study of the formation of oospores in *Phytophthora* has been an interesting subject for investigation and several workers have been on this problem though the last word has not yet been written. Oospore formation is influenced by various factors such as the temperature at which the organism is grown, the medium on which it is grown, the age of the isolates and lastly the innate character of the isolate itself. There are some species in which the sexual bodies have not been recorded yet. The development of the sexual bodies in the *Phytophthora palmivora* group has been investigated by different authors and divergent views have been expressed about the causes leading to this phenomenon. One school represented by Ashby (1928-29) and Lester Smith (1927) is of the opinion that the oospore formation is brought about by some sort of biochemical stimulation of one strain by the other. Lester-Smith states that the production of oospores "in mixed cultures is due to the influence of one vegetation on the other acting through its effect on the medium or on certain constituents of the medium". Gadd (1924), Thompson (1929), Narasimhan (1930), Leonian (1931), Venkatarayan (1932) and Uppal and Desai (1939) on the other hand believe in the heterothallic nature of the isolates of this species. Some prefer to call the isolates 'Plus' and 'Minus' strains while Narasimhan and Leonian who have traced the origin of the hyphae producing the antheridia and oogonia, call the isolates male and female. Tucker (1931) is not convinced of the heterothallic nature of the isolates.

The present investigations carried out with 25 isolates of *Phytophthora*, the bulk of which were obtained from this province and a few from outside the province, have shown that all of them fall into two distinct groups based on their capacity to produce oospores in paired cultures. The isolates of one group form oospores when mixed with isolates from the other. Different combinations of the members of the two groups have been made and the results have been consistent throughout. In the light of present knowledge this behaviour can only be attributed to heterothallism within the same species.

It has moreover been found that in all cases the oogonia and antheridia are borne on different hyphae and never on the same hypha. This again is an indirect evidence of heterothallism. Narasimhan (1930) and Leonian (1931) have claimed to have traced the antheridia and oogonia to different thalli, which is a direct proof of heterothallism of the isolates studied.

It has also been found that some isolates have gradually lost their sexuality in course of time when grown on media. The deterioration of the capacity for oospore formation seems to be attributable to gradual loss of the sexual vigour of the strains through continued growth on agar media.

The behaviour of some of the isolates which formed oospores in single strain cultures originally but later failed to produce them is intriguing. This can be explained away in two ways. It is possible that the original culture was itself a mixed one as might be expected when the fungus is isolated by tissue cultures and the original host had been infected by both the strains. An observation made by the writers in 1946 favours this view. In 1946 a fresh isolate was obtained from breadfruit by tissue culture. In the first generation abundant oospores were formed. From this culture single hyphal tips were transferred to agar slants. Oospores failed to form in these secondary cultures indicating that the original isolate was mixed. But McRae (1917) recorded oospores in single sporangial isolates of *P. meadii*. It has been noticed by several investigators that the cultures supplied to them from Coimbatore did not develop oospores. Even in India the same phenomenon was experienced. McRae had observed oospores on *Hevea* fruits also. This could be explained on the assumption that the strain was originally homothallic but during the growth of the cultures on agar media for a number of generations dissociation took place and the loss of one sexual factor resulted therefrom. It is, however, interesting to note that the isolates from *Agave*, *Hevea* and *breadfruit*, which were originally reported to be forming oospores in single strain cultures and have now become non-oospore-forming, fall into the same sexual thallus group which produces oospores in paired cultures with individuals of the same complementary group. No isolate belonging to the opposite group isolated in this province has ever been known to form oospores in single strain cultures. Leonian (1931) obtained seven dissociants from a culture of *P. parasitica*. Of these six behaved as females and one as a male. He has also obtained other dissociants which could be termed neutral since they failed to form oospores with either of the male or female isolates. Even in the heterothallic strains under study the sex vigour has been lost owing to long culturing on agar media or formation of neutral dissociants. Thus members of the *P. palmivora* group behave as homothallic, heterothallic or neutral strains though with continued growth on agar media many of the strains may become neutral. This change is observable in both groups of complementary strains. Therefore, for the correct identification of the isolates fresh cultures are essential.

These investigations have been helpful in deciding the taxonomic relationships of the isolates. The isolates studied have been variously classified at present. Tucker (1933) and Leonian (1934) have suggested certain revisions of the classification of *Phytophthora* species. Tucker has merged together *P. palmivora*, *P. areca*, *P. faberi* and *P. meadii* into one species under *P. palmivora*. Leonian believes that *P. mexicana* Hots and Hart, *P. parasitica*, *P. parasitica* var. *rhei* God., *P. parasitica* var. *nicotianæ* Tucker, *P. terrestris* Sherb., *P. melongenæ* Saw. and *P. symmetrica* Sid. also should be brought under *P. palmivora*. The isolates under investigation are usually classified as follows: *P. palmivora* on coconut, palmyra, citrus and betel vine, *P. areca* on arecanut and tomato, *P. faberi* on cocoa, *P. meadii* on Hevea, *P. parasitica* var. *nicotianæ* on tobacco, *P. sp.* (not determined) on *Clerodendron*, breadfruit, *Spondias*, *Jatropha*, Jak and *Colocasia* and *P. parasitica* on Agave.

The basis for specific differentiation has been morphological features of the hyphæ, sporangia, chlamydospores and oospores, when formed. Pathogenicity has also been utilized for separating species. Studies on this genus have shown (Tucker, 1931 and Leonian, 1934) that the morphological characters of the mycelium, sporangia and chlamydospores are so plastic as to be of little use in specific differentiation. Leonian (1934) says that "pathogenicity is of still less value, the shape and size of the chlamydospores altogether useless and that of the sporangia not much better in the taxonomy of species of *Phytophthora*".

The work now recorded has shown that all the isolates under study produce oospores when grown mixed with complementary isolates. All the oospores formed in the various combinations are of the same type and the measurements fall within the range recorded for oospores produced from complementary strains occurring on the same host, e.g. *Areca* (Tyagali)  $\times$  *Areca* (Nilekani) 14.0-31.0  $\mu$ . This feature coupled with the readiness with which oospores are formed in the paired cultures of these isolates brings out the close specific relationship of these isolates. There can be no question of regarding these oospores as of hybrid origin between different species because no constant differences can be made out between these either in the size of the oospores, the nature of the antheridia or any other important character. All these isolates therefore, fall under one species, viz., *P. palmivora* Butler.

This species has a wide host range. Not less than sixteen species of host plants have so far been recorded from S. India. It is heterothallic but the present state of our knowledge suggests the possibility of some isolations

being homothallic. Two distinct sexual strains—the plus and the minus—are seen and the collections in our possession are classified under the two heads as follows

Plus	Minus
Areca (Nilekani)	Areca (Tyagali)
Betel vine	Palmyra
Citrus I	Coconut
Clerodendron	Cacao
Colocasia	Rubber
Jak	Breadfruit
Tomato	<i>Spondias</i>
	Agave
	Tobacco
	Citrus II
	<i>Jatropha</i>

The grouping of the isolates into the "Cacao" and "Rubber" groups adopted by Gadd and followed by Ashby is rather confusing. The same host has been found to be affected by both the strains. For example the "Cacao" isolate from Ceylon really behaves like an isolate belonging to the "Rubber" group. Ashby has also found that different isolates from cacao and coconut may fall into different groups. Therefore, the naming of the groups according to the host is misleading.

Uppal and Desai (1939) have obtained two complementary isolates Tyagali and Nilekani—form the same host viz *Areca*. Tyagali behaves like the isolates from palmyra and coconut in Madras. Butler (1910) has recorded that *P. palmivora* affects arecanuts causing bud-rot. It is possible that the Tyagali strain represents the coconut strain (minus) which has become parasitic on *Areca* in that locality. Coconut is also infected by both the strains. *Citrus* in India is also parasitised by both. When such mixed or combined infections occur in nature on the same host plants, there is every possibility of oospores developing as has been recorded in breadfruit and *Hevea* rubber. This represents one of the methods of 'over-summering' of the fungus under tropical conditions obtaining in South India. Whether sexual reproduction gives rise to new races is a matter for further investigation.

The plant pathologist has to consider the significance of these results. These facts bring out the necessity for vigilance on his part concerning the occurrence of *Phytophthora palmivora* on a variety of hosts some economically important and others of no importance. Inasmuch as this species

has a wide host range, the passage from one host to another is easy under favourable conditions. Further, the part played by the non-crop-hosts in the survival of the pathogen, the formation of sexual bodies when the same host becomes infected by the two sexual strains and the possible production of new strains as a result of sexual reproduction cannot be overruled. The parasitism of *P. palmivora* being by no means specialised, every record of this species on any host has to be considered as a source of potential danger to the crop plants known to serve as hosts of this species in the locality.

#### ACKNOWLEDGEMENTS

We are grateful to Dr B. N. Uppal, Plant Pathologist, Bombay, and Mr M. J. Narasimhan, Director of Agriculture, Mysore, for having kindly sent the cultures of strains of *P. arecae*.

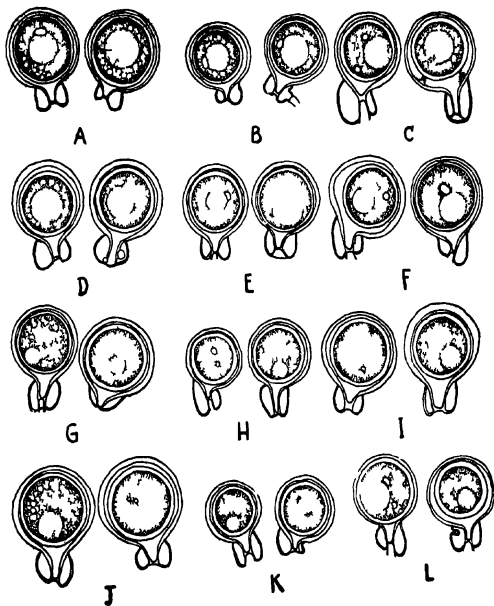
#### SUMMARY

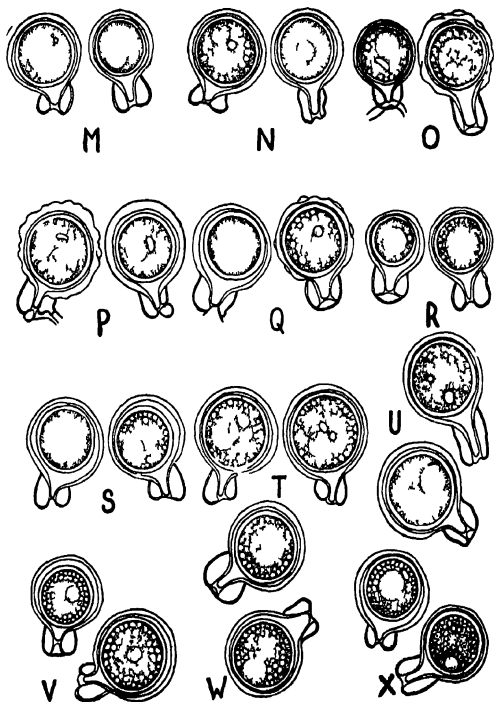
The formation of oospores in paired cultures of twenty-five isolates of *Phytophthora* was studied. These isolates fall into two main groups—the “plus” and the “minus” and the members of one group form oospores when paired with members of the other group. Some of the isolates were found to lose their sexual capacity with continued cultivation on agar media. Fresh isolates form oospores quickly with complementary strains.

All these isolates belong to *P. palmivora*, Butl. The other species of *Phytophthora*, viz., *P. arecae*, *P. meadu*, *P. faberi* and *P. parasitica* var. *nicotiana*—are to be merged in *P. palmivora* as they are found to be morphologically similar and do not exhibit any constant and reliable differences from *P. palmivora* and readily form oospores when paired with it. This species is heterothallic, but homothallism has been reported to have been noticed in some isolates.

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# EXPLANATIONS OF PLATES

All figures were drawn with the aid of an Abbe camera lucida at a uniform magnification of  $\times 680$ .

Plates VIII and IX. Sexual bodies produced in paired cultures of 'plus' and 'minus' strains of *Phytophthora palmivora* Butler

## PLATE VIII

- A. Jak  $\times$  Jatropha  
 B. Jak  $\times$  Palmyra  
 C. Jak  $\times$  Hevea  
 D. Jak  $\times$  Citrus II  
 E. Jak  $\times$  Agave  
 F. Jak  $\times$  Spondias  
 G. Tomato  $\times$  Citrus II  
 H. Tomato  $\times$  Jatropha  
 I. Tomato  $\times$  Cocoa  
 J. Tomato  $\times$  Hevea  
 K. Tomato  $\times$  Tobacco  
 L. Tomato  $\times$  Agave

## PLATE IX

- M. Tomato  $\times$  Coconut  
 N. Citrus I  $\times$  Spondias  
 O. Citrus I  $\times$  Jatropha  
 P. Citrus I  $\times$  Palmyra  
 Q. Citrus I  $\times$  Citrus II  
 R. Breadfruit (alone)  
 S. Clerodendron  $\times$  Hevea  
 T. Clerodendron  $\times$  Spondias  
 U. Be el vine I  $\times$  Spondias  
 V. Be el vine II  $\times$  Spondias  
 W. Colocasia  $\times$  Spondias  
 X. Areca  $\times$  Spondias

## EMBRYOGENY OF *ISOTOMA LONGIFLORA* PRESL.

By S B KAUSIK AND K SUBRAMANYAM

(Department of Botany Central College Bangalore)

Received June 25 1947

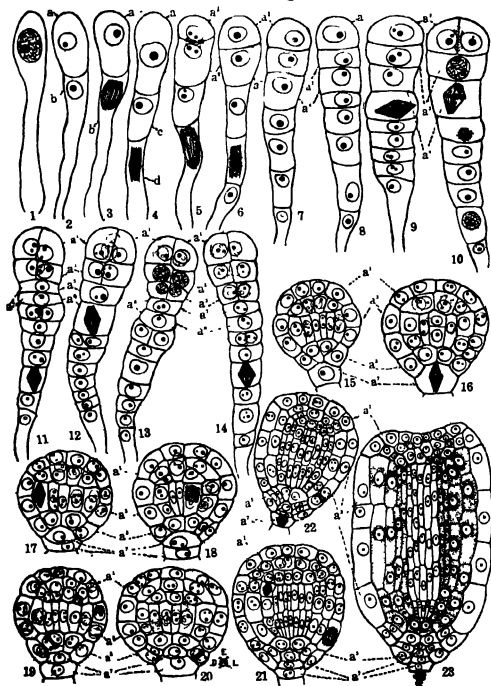
(Communicated by Prof L Narayan Rao F A Sc)

In a previous paper of ours (Kausik and Subramanyam 1945 *a*) a detailed account of the development of the male and female gametophytes and endosperm formation in *Isotoma longiflora* Presl has been given. The present paper deals with the development of the embryo in this plant. During this study a single case of polyembryony was met with and this has been separately described (Kausik and Subramanyam, 1946).

Fixation of the material was done in Formalin Acetic Alcohol and the sections were stained in Heidenhain's iron-alum-haematoxylin with eosine as counterstain.

The fertilised egg elongates rapidly and becomes tubular with the nucleus situated at the apex (Fig 1). The first division of the fertilised egg takes place after the endosperm has passed through its initial development. It divides in a transverse manner cutting off a primary embryonal cell (Fig 2 *a*) and a primary suspensor cell (Fig 2 *b*). Of these it is the primary suspensor cell (Fig 2 *b*) that divides first by a transverse wall to form a middle cell *c* and a basal cell *d* (Fig 4). Thus a three-celled proembryo is formed. In this respect *Isotoma longiflora* resembles *Campamula patula* L (Soueges, 1936), *Lobelia amana* (Hewitt, 1939), and *L. trialata* Buch-Ham (Kausik and Subramanyam, 1945 *b*). In *L. syphilitica* L (Crete, 1938) and *Cephalostigma Schumperi* Hochst (Kausik and Subramanyam, in Press), however, it is the primary embryonal cell that divides first. In the three-celled proembryo the middle and basal cells (Figs 3, 4 and 5) usually divide by further transverse walls, thus adding to the length of the suspensor (Figs 6 to 14).

The primary embryonal cell *a* now divides first by a transverse wall (Fig 5) cutting off an apical cell *a*<sup>1</sup>, which does not divide further until longitudinal divisions begin, and a second embryonal cell *a*<sup>2</sup>. The second cell of the proembryo *a*<sup>2</sup> divides by a transverse wall to form cells *a*<sup>3</sup> and *a*<sup>4</sup> (Figs 7 to 9). At about this stage one of the suspensor cells, usually in the upper region of the filamentous proembryo, divides by a vertical wall (Figs 9 and 10) to form two cells, which are characteristically seen in the early stages of embryogeny (Figs 11 to 14). A similar feature is seen in *Jasione montana*



FIGS. 1-23. *Isotoma longiflora* Presl.—Stages in the development of the embryo. For explanation see text. Figs. 1-20.  $\times 1260$ . Figs. 21-23.  $\times 900$  (Original magnifications given here, but figures have been reduced to half in reproduction).

Linn (Souéges, 1938), *Lobelia amana* (Hewitt, 1939) and *L. trialata* (Kausik and Subramanyam, 1945b). In *Lobelia syphilitica* (Crete, 1938) and *Cephalostigma Schumperi* (Kausik and Subramanyam, in Press) a group of four cells is formed by the activity of one of the suspensor cells. In *Campanula patula* (Souéges, 1936) more than four cells are formed in this region.

The cells  $a^1$  and  $a^2$  of the filamentous proembryo divide by two sets of longitudinal walls (Figs 10 to 13) at right angles to each other, thus resulting in two tiers of cells with four cells in each tier. Almost simultaneously with these divisions cell  $a^1$  divides by a transverse wall producing cells  $a^3$  and  $a^4$  (Figs 10 and 11). Cell  $a^2$  then divides by two vertical walls so that three tiers of cells are now formed in the terminal region of the proembryo (Fig. 14) with four cells in each tier. Cell  $a^4$  also divides by a transverse wall adding two more cells  $a^5$  and  $a^6$  to the terminal region (Figs 12 to 14). Thus in this region five tiers of cells can be made out viz.,  $a^1$ ,  $a^2$ ,  $a^3$ ,  $a^5$  and  $a^6$ , the three upper tiers having four cells each and the lower two having only a single cell each. Of these five tiers it is only the first four tiers that actually take part in the formation of the various regions of the embryo.

In the distal tier  $a^1$ , anticlinal divisions occur followed by periclinal divisions to form the dermatogen in this region (Fig. 15). The first anticlinal divisions in this tier can be traced in the various stages upto the formation of the mature embryo (Fig. 23). The next division in tier  $a^1$  is tangential cutting off a dermatogen peripherally from a group of inner cells (Fig. 15). The inner cells divide longitudinally separating the future periblem from the plerome. Both longitudinal (Fig. 18) and transverse divisions (Fig. 17) occur in the further development of the plerome and periblem (Figs 18 to 23).

When the primary body regions are differentiated in the first two tiers, the third tier  $a^3$  develops into a semicircular layer of cells at the base of the embryo. To start with, this layer is made up of four cells (Figs 15 to 17), but subsequently forms about 8 cells (Figs 22 and 23) by further oblique divisions (Figs 18 to 21). The innermost two cells of this group take part in the completion of the periblem (Fig. 23). The remaining cells of this layer help to complete the dermatogen and the root cap. The single cell of tier  $a^2$  does not divide any further until the embryo is rather well developed and spherical in shape. Then it divides by a transverse wall (Fig. 16) forming a proximal and a distal cell (Figs 17 to 20). Both these cells divide transversely (Figs 21 to 23) forming a part of the root-cap which is also increased on all sides from tier  $a^3$  and also by extra cells cut off from the dermatogen in tier  $a^1$ . According to Hewitt (1939) however, in *Lobelia amana* the cells of the proximal row, cut off from this tier, do not divide again, but the cells

of the distal row divide transversely separating the periblem from the dermatogen. He further states that the cells of the proximal row become a part of the root cap. In a mature embryo (Fig 23) the body regions can easily be assigned to the primary tiers viz  $a^1$ ,  $a^2$ ,  $a^3$  and  $a^7$  which are clearly recognizable in the early stages of embryogeny. Thus from tier  $a^1$  arise the cotyledons, the stem tip forming in the notch between them, from tier  $a^2$  the hypocotyl is formed with its central row of long and narrow plerome cells, the outer zone of much larger periblem cells (the region shown dotted in Fig 23) and the outermost layer of dermatogen. From tier  $a^3$  the completion of the periblem and the organisation of a part of the root cap take place, and lastly from tier  $a^7$  the rest of the root cap is formed.

## ACKNOWLEDGMENT

We are thankful to Dr L. N. Rao, Professor of Botany, Central College, Bangalore, for kind encouragement during the course of the present study.

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## THE NEWLY HATCHED LARVA OF *PERICLIMENES* (*ANCYLOCARIS*) *BREVICARPALIS* (SCHENKEL)

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(From the University Zoological Research Laboratory, Madras)

Received April 26, 1947

(Communicated by Prof S G M Ramanujam, F A SC)

### INTRODUCTION

THE occurrence of *Periclimenes* (*Ancylocaris*) *brevicarpalis* in association with *Stoichactis giganteum* (Forsk.) at Krusadai Island has been recorded by Gravely (1927) in his study on the littoral Decapod fauna of the Island. Kemp (1922) has collected the species from the giant sea-anemone *Discosoma* at Port Blair. The genus *Periclimenes* comprises of a large number of species about the larval history of which very little is known. Gurney (1936 and 1938) has described the larval stages of the following species of *Periclimenes* (sub-genus *Ancylocaris*), e.g. —*calmani*, *americanus*, *agag*, *diversipes* and *grandis*. As the larval history of *Periclimenes* (*Ancylocaris*) *brevicarpalis* is not known it was thought useful to describe the newly hatched larva.

During a brief stay at the Krusadai Biological Station in the Gulf of Manaar, in March 1947, the author collected numerous specimens of *Periclimenes brevicarpalis* including a good number of berried forms from Kundugal Point where *Stoichactis giganteum* occurs in abundance. Generally each anemone shelters a male and a female under the exposed flattened tentacle-bearing region, and even when the anemone contracts they very seldom make an attempt to swim away. Though the prawns are transparent, as observed by Gravely (1927) certain regions of the body are coloured very prominently as described by Kemp (1922).

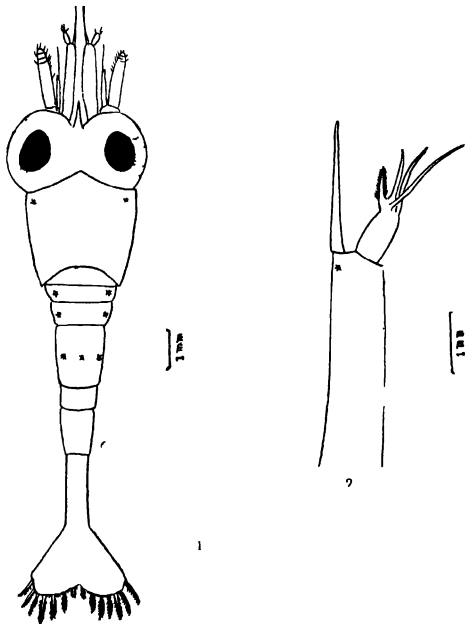
From a very close observation of a large number of forms, the present author while confirming Gravely's observation is also led to the conclusion that this prominent colouration resembling that of broken shells helps the prawns to escape observation when the anemone contracts and withdraws into its burrow leaving the prawns exposed.

### DESCRIPTION OF THE NEWLY HATCHED LARVA

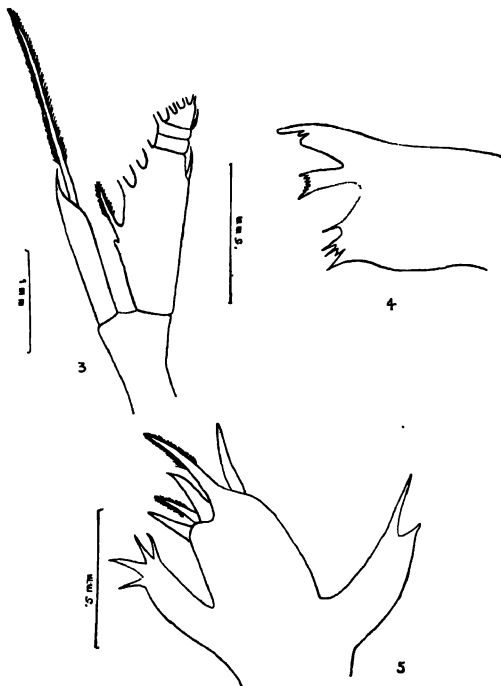
The eggs of some of the berried females on microscopic examination seemed to be in an advanced stage of development, and were therefore kept under observation in the laboratory aquaria. Aeration of the eggs

*Newly Hatched Larva of Perichimenes brevicarpalis (Schenkel)* 169

appears to be effected by the side to side rocking movement of the entire animal aided by the gentle movement of the pleopods. The eggs measuring 0.41 mm to 0.31 mm in diameter hatched out in the evening. The newly



FIGS 1 & 2



FIGS. 3-5

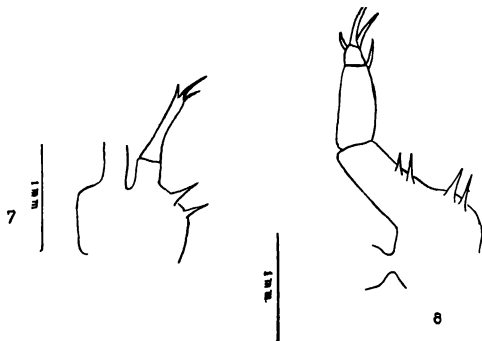


hatched larvæ, 1.65–1.7 mm. in length (Fig. 1), are very active in their movement and phototactic in behaviour.

*Coloration.*—The larva is perfectly transparent. Two orange coloured chromatophores are present on the sides of the anterior region of the carapace. On either side of the first abdominal somite is present a shining yellow chromatophore. Chromatophores of the same colour and disposition are present on the second and third abdominal somites, the latter carrying a median dorsal chromatophore in addition. Similar chromatophores are present on the endopods of the second and third maxillipedes.

*The eyes.*—The eyes are sessile.

*Antennule* (Fig. 2).—The peduncle is unsegmented. The inner flagellum is in the form of a single large seta. The outer flagellum is represented by a small papilla bearing at its tip three aesthetes and a short plumose seta.



Figs 7 8

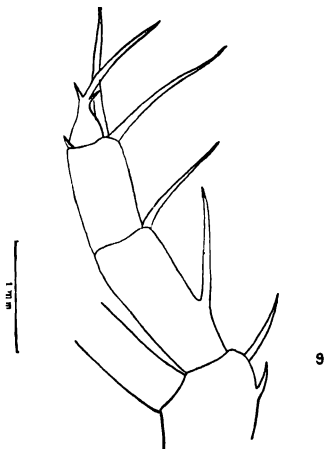
On the peduncle, at the base of the inner flagellum, is a shining golden yellow stellate chromatophore

*Antenna* (Fig 3)—The peduncle is unarmed. The inner flagellum is cylindrical unsegmented and carries a spine and a long plumose seta. The inner flagellum inclusive of the spine is about two thirds the length of the scale. The scale is divided into three distinct segments and carries a small papilla and nine plumose setae along its inner margin. There are two spines on the outer margin of which the proximal one is longer.

*Mandible* (Fig 4)—The mandible is clearly marked off into the incisor and molar regions. The incisor part carries three blunt teeth and the molar part has five pointed spines. In between the incisor and the molar regions is a blade which is serrated at the tip.

*Maxillule* (Fig 5)—Endopod of maxillule is bilobed and carries a small seta. The proximal masticatory process is narrow and has four setae. The distal process is provided with two plumose setae and three pointed spines.

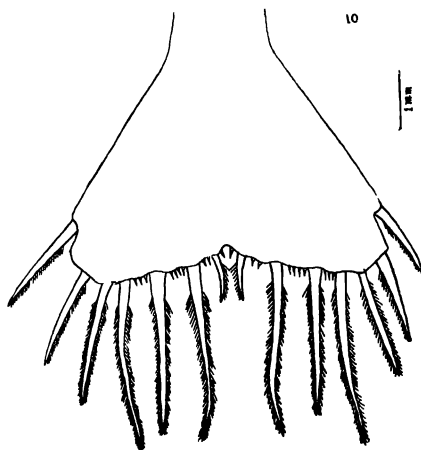
*Maxilla* (Fig 6)—There are three masticatory processes on the protopodite. The proximal process carries two setae, the middle and the distal



processes bear one and two setæ respectively. The endopodite is not bilobed and is tipped with a single seta. The scale carries four plumose setæ.

*Maxillipede 1* (Fig. 7)—Basipodite is not protuberant and carries two spines. Endopodite is bisegmented and carries three terminal spines. The exopodite is unsegmented and carries four setæ.

*Maxillipede 2* (Fig. 8)—Coxopodite is unarmed. Basipodite has two spines. The endopod is distinctly three segmented. The basal segment on its inner side carries two spines. Another spine is placed at the base of the third segment. The third segment carries two long terminal setæ and one small spine on its outer margin. Exopodite is unsegmented and is similar to that of the previous appendage.



*Maxillipede 3* (Fig 9)—Basipodite carries two spines. The endopodite is of three distinct segments of which the first and second bear on their inner margin one spine each. At the base of the second segment on its inner margin is another spine. Dactyl ends in a single strong terminal spine and carries two small spines, one on its outer side and another on its inner side. At the base of the terminal segment on its inner side is a small spine.

*Telson* (Fig 10)—The telson is slightly concave on the ventral side. The usual seven pairs of spines are present. The fourth spine is slightly

*Newly Hatched Larva of Periclimenes brevicarpalis (Schenkel) 175*

longer than the sixth. The innermost is the smallest and is enclosed in a sheath in the embryonic condition.

# REMARKS

Gurney (1938) has summarised the larval characters of the genus *Periclimenes* (*Ancylocaris*), based on the larval stages of *grandis*, *agag*, *calmani*, *diversipes* and *americanus*. Gurney (1938) has also emphasised the fact that the larval characters mentioned are applicable in general to the larvæ of the genus *Periclimenes* exception being made to *Periclimenes* (*Ancylocaris*) *diversipes*. The larva of *Periclimenes* (*Ancylocaris*) *brevicarpalis* shows some interesting features in comparison to the other species.

Name of Species	Appendages			
	Maxillule	Maxilla	Maxillipede I	Maxillipede III
P (A) agag	endopod bilobed	endopod has a basal lobe. Scale has four plumose setæ	endopod not distinctly segmented. Basal protuberant	Dactyl ends in two strong terminal spines
P (A) diversipes	endopod not bilobed	endopod has no basal lobe. Scale has five plumose setæ	endopod unsegmented, basal slightly protuberant	Dactyl ends with a single strong terminal spine
P (A) grandis	endopod bilobed	Basal lobe present in the endopodite. Scale has four plumose setæ	endopod not distinctly segmented	Dactyl ends in two long sub-equal terminal spines
P (A) brevicarpalis	endopod bilobed	Basal lobe of endopod absent. Scale has four plumose setæ	Basipodite not protuberant. Endopodite bisegmented	Ends in a single strong terminal spine

It was not possible to study the subsequent stages owing to the shortness of the author's stay at the station, nevertheless these few observations are recorded here in the hope that the opportunity for continuing the work will be available in the near future.

# ACKNOWLEDGEMENT

I wish to express my gratitude to Dr. C. P. Gnanamuthu, M.A., D.Sc., F.Z.S., Director, University Zoology Laboratory, Madras, for his help and criticisms throughout the course of this work.

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## EXPLANATION OF FIGURES

FIGS 1-10 The newly hatched larva of *Periclimenes (Ancylocaris) brevicarpalis* and its appendages—Fig. 1 The newly hatched larva Fig. 2 Antennule Fig. 3 Antenna Fig. 4 Mandible Fig. 5 Maxillule Fig. 6 Maxilla Fig. 7 Maxillipede 1 Fig. 8, Maxillipede 2 Fig. 9 Maxillipede 3 Fig. 10 Telson

## ERRATA

*Vol XXVI, No 2, August 1947, Sec B*

On page 69, third line from bottom, "0 028 gm. of nitrogen" *should read as* "0 28 gm of nitrogen"

The caption of the Y-axis of text-figures 2 and 4 on pages 70 and 72 should read as *macroconidia* and not *microconidia*

## ERRATUM

*Vol XXVI, No 2, August 1947, Sec B*

Plate IV —read 1, 4 and 7 in place of 3, 6 and 9, and 3, 6 and 9, in place of 1, 4 and 7.



# COPEPODS OF THE WEST HILL SEA\*

BY P K JACOB AND M. DEVIDAS MENON

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(Communicated by Prof. Ben Charan Mahendra, D Sc, F.Z.S., F.A.S.C.)

CONTENTS		PAGE
I	INTRODUCTION	177
II	PHYSICAL FEATURES AND CLIMATIC AND HYDROGRAPHICAL CONDITIONS OF THE COAST	178
III	COPEPODS OF THE WEST HILL AREA	180
IV	FLUCTUATIONS OF COPEPODS IN GENERAL IN THE WEST HILL SEA FOR THE QUINQUENNIAL 1939-40 TO 1943-44	181
V	SEASONAL FLUCTUATIONS OF SIX IMPORTANT GENERA OF COPEPODS OF THE WEST HILL SEA FOR THE YEAR 1945-46	184
	<i>Oithona</i>	185
	<i>Paracalanus</i>	185
	<i>Acartia</i>	185
	<i>Temora</i>	185
	<i>Euterpina</i>	185
	<i>Corycaeus</i>	185
VI	FLUCTUATION IN THE COPEPOD POPULATION OF THE WEST HILL AREA WITHIN THE YEAR 1945-46 AND ITS CORRELA- TION WITH DIATOMS AND HYDROGRAPHIC AND METEORO- LOGICAL FACTORS	185
VII	COPEPOD FLUCTUATION IN RELATION TO WEST COAST FISHERY FOR THE QUINQUENNIAL 1939-40 to 1943-44	188
VIII	COPEPODS AND FISHERIES	192
IX	COPEPODS AND MACKEREL FISHERY	192
X	DISCUSSION	192
XI	ACKNOWLEDGEMENTS	193
XII	REFERENCES	193
APPENDIX—		
	(Fishes of the West Hill Area. found to feed on Copepods)	194

## I. INTRODUCTION

THE importance of Copepods in plankton cannot be over-emphasized. As pointed out by Johnstone Scott and Chadwick (1924), they "are ubiquitous

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and abundant and of prime economic importance, in the respect that they are plentiful source of food both for many pelagic animals like Herring and for hosts of small fishes" They constitute a major group of organisms in the zoo-plankton, contributing (as found by us) as much as 75 to 90 per cent of the total population of the planktonic organisms in the West Hill area of the Arabian Sea and forming "one of the essential links in the food chain of the sea" (Clarke, 1939) They occur almost throughout the year in this area also, just as in the sea off the Trivandrum Coast (Menon, 1945) and in the Madras Coastal waters (K S Menon, 1931)

Although Copepods occur throughout the year in the West Hill plankton, they undergo seasonal fluctuations in number, owing to the varying population of diatoms, which depends upon the hydrographic and meteorological conditions The present communication is an attempt to trace these relationships for the five years, beginning from July 1939 and ending with June 1944<sup>1</sup> A list of the genera and species of Copepods abounding in the West Hill area is recorded, and the seasonal fluctuations of six important genera of Copepods have been traced For one year (1945-46) the fluctuation has been studied in greater detail with special reference to its correlation to diatoms and physical factors In order to add to our knowledge of the food chain of the sea, the authors have tried to correlate the Copepodan abundance with the amount of plankton-feeding fishes in general and the Indian Chub Mackerel (*Rastrelliger kanagurta*) in particular, landed on the Calicut Coast

## II PHYSICAL FEATURES AND CLIMATIC AND HYDROGRAPHICAL CONDITIONS OF THE COAST

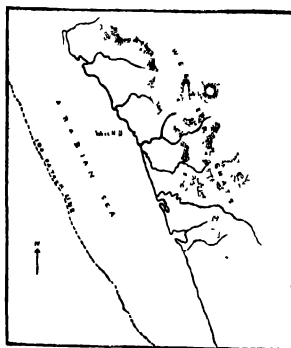
### (a) Physical Features

The West Coast of Madras is characterised by a narrow strip of low-lying land between the high mountains of Western Ghats, rising to an average height of 5,000 feet, and the Arabian Sea (Fig 1) Naturally, the rivers flowing into the sea are very short The descent from the mountains being steep, these rivers flow in great torrents, carrying rich debris into the sea Perhaps this feature is responsible for the wide Continental Shelf fifty miles broad here (Raj, 1931)

### (b) Climatic Conditions

**Rainfall**—The West Coast of Madras receives rain from two monsoons The greater part is obtained from the South-West Monsoon which sets in

<sup>1</sup> The computation is in accordance with the Fisheries year, beginning in July of one calendar year and ending in June of the following year



TEXT-FIG. 1 Map of the Malabar Coast (Scale 1 inch = 28 miles)

May or June and ends in August. During these months, the sea is very turbulent and turbid. The North-East Monsoon occurs in October and November (Fig. 4). The average rainfall at West Hill is  $107.5^{\circ}$ .

(c) *Hydrographical Conditions*

*Specific Gravity.*—The lowest specific gravity is in the months of June, July and August. In all the other months except in November, in which month there is a slight fall, the specific gravity curve maintains an average high level (Fig. 4) (Chidambaram and Menon, 1945)

*Surface Temperature.*—According to Sewell (1925-29), the surface temperature of Indian seas directly depends upon the monsoon. Our investigations confirm this statement. The lowest surface temperature is noticed to occur in the months of June to September, *i.e.*, during the South-West Monsoon. The highest peak is reached in the months of March, April and May. The little fall in the surface temperature in the months of December and January is due to the North-East Monsoon and the currents (Fig. 4) (Chidambaram and Menon, 1945).

## III. COPEPODS OF THE WEST HILL AREA

The following genera and species form the bulk of the Copepod group:—

Genus *Canthocalanus* A. Scott.

1. *Canthocalanus pauper* (Giesbrecht).
2. *Calanus finmarchicus* (Gunnerus).

Genus *Eucalanus* Dana.

3. *Eucalanus crassus* Giesbrecht.
4. *Eucalanus suberasus* Giesbrecht.
5. *Eucalanus attenuatus* Dana.

Genus *Undinula* A. Scott.

6. *Undinula vulgaris* (Dana)

Genus *Paracalanus* Boeck.

7. *Paracalanus parvus* Giesbrecht.
8. Genus *Pseudocalanus* Boeck

Genus *Rhincalanus* Dana

9. *Rhincalanus nastus* Sars.

Genus *Pseudodiaptomus* Herrick.

10. *Pseudodiaptomus annandalei* Sewell.

Genus *Temora* W Baird.

11. *Temora discaudata* Giesbrecht
12. *Temora longicornis* (Muller).

Genus *Centropagus* Kroyer

12. *Centropagus furcatus* Dana.
13. *Centropagus tenuiremis* Thompson and Scott.

Genus *Labidocera* Lubbock

14. *Labidocera acuta* (Dana).

Genus *Metridia* Boeck.

15. *Metridia lucens* Boeck.

Genus *Candacia* Dana.

16. *Canadacia truncata* Brady.

Genus *Pontella* Dana.

17. *Pontella danae* Giesbrecht
18. *Pontella securifer* Brady.

Genus *Acartia* Dana.

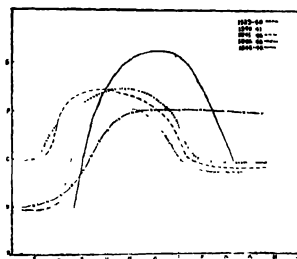
19. *Acartia erythrea* Giesbrecht.
20. *Acartia discaudata* Giesbrecht.

- Genus *Oithona* Baird  
 21 *Oithona rigida* Giesbrecht  
 22 *Oithona plumifera* Baird  
 Genus *Clytemnestra* Dana  
 23 *Clytemnestra rostrata* (Brady)  
 Genus *Microsetella* A. Scott  
 24 *Microsetella rosea* Dana  
 Genus *Onca* Phillipi  
 25 *Onca venusta* Phillipi  
 26 *Onca confifera* Giesbrecht  
 27 *Onca ornata* Giesbrecht  
 Genus *Sapphirina* J. W. Thomson  
 28 *Sapphirina ovatolanceolata* Dana  
 29 *Sapphirina nigromaculata* Claus  
 Genus *Isias* Boeck  
 30 *Isias clavipes* Boeck  
 Genus *Corycaeus* Dana  
 31 *Corycaeus elongatus* Claus  
 32 *Corycaeus venustus* Dana  
 33 *Corycaeus furcifer* Claus  
 Genus *Euterpina* Norman  
 34 *Euterpina acutifrons* (Dana)

IV FLUCTUATIONS OF COPEPODS IN GENERAL IN THE WEST  
 HILL SEA FOR THE QUINQUENNIAL 1939-40 TO 1943-44  
 (Text Fig. 2)

1939-40.—In July 1939, the Copepods occurred scarcely, but in August and September there was a steady rise, so that the 'Plenty'<sup>1</sup> stage was reached in September and even exceeded in October. However, it was during November that the maximum was reached, which was half way between the 'Plenty' and the 'Swarm' stages. From December onwards there was a steady fall, a reversal of the rise noticed in August and September. In January the level fell to the 'Common' stage and from February onwards a slightly lower level than this was maintained. Comparing the Copepodan abundance of this year with those of the other four years,

<sup>1</sup> In the rough quantitative estimation of planktonic organisms made in the West Hill Biological Station, J. Hornell applied the following terms in the increasing order 'Rare', 'Few', 'Common', 'Plenty' and 'Swarm'. This quantitative analysis is being still followed.



TEXT-FIG. 2 Chart showing the Annual Fluctuations of Copepods of the West Hill Sea for the quinquennium 1939 to 1944. Months are shown on the horizontal axis and the frequency of copepods on the vertical. *J, A, S, O, N, D, J, F, M, A, M, J*, months from July to June. *R* = Rare. *F* = Few. *C* = Common. *P* = Plenty. *S* = Swarm.

the Copepods seem to have maintained a fairly high level for more than an average length of time, which phenomenon may be attributed to the normal climatic and hydrographical features of this year favouring the abundance of diatoms (*i.e.*, the food for Copepods) present in the sea, especially at the beginning of the year.

1940-41.—This year was not a favourable one like the previous one for Copepods. Almost up to the month of September, the Copepods were 'Few' in plankton. It was during this month that the quantity of Copepods increased towards the 'Plenty' stage, but it never exceeded this stage. The decline began earlier this year, *i.e.* in December, and by January the level fell below the 'Common' stage from which it never rose again. On the whole, this was a very poor year for Copepods, probably due to such adverse hydrographical conditions as high temperature, specific gravity, etc. (Chidambaram and Menon, 1945).

1941-42.—During this year, conditions seem to have been quite fair for Copepods. This year's fluctuation simulates that of 1939-40 with the minor difference that the Copepods flourished better until the month of October and were less abundant afterwards. The fair weather with a large number of sunny days seems to have had a beneficent effect on the copepodan abundance.

1942-43 —The fluctuation curve of Copepods takes a different aspect this year from that of the other years under review. The rise to the 'Plenty' stage is reached rather late in November, but once it has risen to this stage it is maintained uniformly throughout the rest of the year. The unusually prolonged monsoon and the very low surface temperature of this year account for this peculiar curve (Chidambaram and Menon, 1945).

1943-44 —The late appearance of Copepods in the sea and the unprecedented rise in their abundance characterize this year. The 'Swarm' stage was maintained in December and January. In February the fall was as steep as was the rise in September. The diatom abundance alone seems to have been responsible for the swarming of Copepods this year.

The fluctuation curves for the three years 1939-40, 1940-41 and 1941-42 resemble each other in showing a rise in abundance during the months of July and August, a high level having been reached in all cases in September and maintained during October, November and December, this being followed by a low level in January. The differences from such a frequency curve noted in the years 1942-43 and 1943-44 are accounted for by climatic changes and variations in the abundance of diatoms which form the food of Copepods.

Regarding the plankton of the Malabar coast, Hornell and Ramaswamy Naidu (1923) reported that Copepods are the dominant crustaceans met with in the plankton. They first appear in quantity in November, increasing to their maximum abundance early in December but suffering no appreciable diminution till January. In March they are reduced to five eighths and they are very scarce from April to August.

The course of Copepodan fluctuation noticed in the present investigation differs from that observed by Hornell and Naidu. According to them, "they appear in quantity in November", whereas a fairly high level was noticed by us to have been reached much earlier (in September) in almost all the years under review. Their statement that in January the number of Copepods are not reduced does not agree with our observations, since in all the first three years the Copepods fell down in number and reached low levels in January.

In 1942 Devanesan and Chidambaram stated that "the entomostracan Copepoda should lead any list of planktonic organisms made in the sea opposite West Hill, for such is its abundance and constancy in its occurrence that it mostly occupies the 'Swarm' stage and falls only to 'Plenty' stage". However, they noted falls in the months of August in 1936-37 and July in 1940-41.

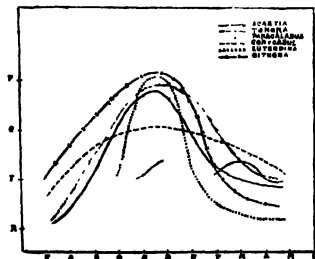
The fluctuation curves of Copepods of the West Hill plankton for the five years under review (Fig. 2) shows that the Copepods as a group are found to be at their lowest ebb from May to September. From this month onwards there is a greater Copepodan activity and the population of Copepods in the plankton increases so that they reach their maxima in the last week of November and early week of December. The fluctuation of Copepods shows unimodal curves indicating a Copepodan abundance for a prolonged period from September to January reaching peaks in December when they assume the 'Swarm' stage in the plankton. From January onwards they begin to decline.

#### V SEASONAL FLUCTUATIONS OF SIX IMPORTANT GENERA OF COPEPODS OF THE WEST HILL SEA FOR THE YEAR 1945-46

As in the Madras plankton, two types of Copepods were recognized in the West Hill plankton according to the nature of fluctuation and appearance that they exhibited:

- (1) Those which closely follow the total Copepodan fluctuation, like *Oithona*, *Paracalanus*, etc
- (2) Those which exhibit short sharp maxima, but are almost altogether absent at other times, like *Euterpina* and *Corycaeus*

This was established from a study (Fig. 3) of the appearance and fluctuations of the following six genera.—



TEXT-FIG. 3. Chart showing the Seasonal Fluctuations of six Important Genera of Copepods of the West Hill Sea, for the year 1945-46. (For explanation of abbreviations, see Fig. 2.)

*Oithona*, *Paracalanus*, *Acartia*, *Temora*, *Euterpina* and *Corycaeus*

*Oithona*.—This is the first Copepod to appear in the plankton in the month of July together with some Crustacean and Copepodan larvæ and post-larval stages. This form is found throughout the year in the plankton and has its maximum period in November and December.

*Paracalanus*.—*Paracalanus* is the most common form in the plankton of this coast. It is very rare in July, but becomes common gradually, reaching its period of maximum abundance in the latter part of October; it continues to be fairly abundant till the end of March. The peak period is from November to January.

*Acartia*.—*Acartia* is continuously present in the plankton and its fluctuation is very similar to that of *Paracalanus*. The peak period, however, is slightly shorter than that of *Paracalanus*.

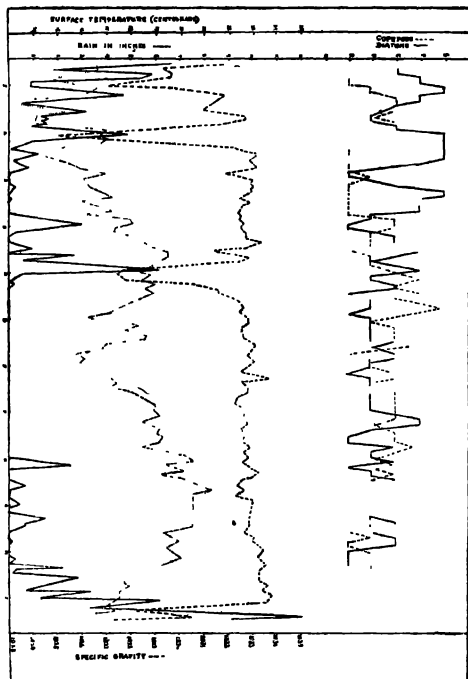
*Temora*.—*Temora*, like the previous three forms, is also constantly present in the general plankton of the West Hill area; but unlike them, it lacks a sharply distinguished peak period, its fluctuation curve being smooth and uniform.

*Euterpina*.—This form makes its first appearance much later than others, in August in the first instance, and it disappears completely from the plankton by the last week of August. In October, it comes up again and is followed by a sharp maximum which falls down considerably in December. Hence onwards only a few of them are found in the plankton.

*Corycaeus*.—*Corycaeus* occur in the plankton in those months when the surface temperature and specific gravity are fairly high. During June, July and August when the specific gravity and surface temperature are low *Corycaeus* is absent.

#### VI. FLUCTUATION IN THE COPEPOD POPULATION OF THE WEST HILL AREA WITHIN THE YEAR 1945-46 AND ITS CORRELATION WITH DIATOMS AND HYDROGRAPHIC AND METEOROLOGICAL FACTORS (FIG. 4)

*July*.—The first ten days in July present a plankton completely devoid of Copepods. Copepods make their appearance on the eleventh day. The same phenomenon was noticed in the plankton of the Madras Coast by Menon (1931). When the diatoms increase, it was seen that Copepods closely follow them (Chidambaram and Menon, 1946). The production of Copepods halts during the period when the diatoms reach the stage of 'Swarms', as was noted in the plankton of the Trivandrum Coast.



TROT-Fig. 4. Chart showing the fluctuation in the Copepod population of the West Hill area within the year 1945-46 and its correlation with Diatoms and hydrographic and meteorological factors. The upper curves represent the frequency of Copepods and diatoms; when the latter three curves show the variations in surface temperature, rainfall and specific gravity during the various months of the year 1945-46

From the third week Copepods tend to increase in numbers as well as in species and with this comes the fall in diatoms. During the last days of the month, both Copepods and Diatoms begin to wane down and assume more or less the same intensity in population.

*August*—The condition at the beginning of the month shows still a downward trend. A slight increase in temperature and salinity and bright sunshine set in and a sudden outburst of diatoms follow. The Copepods follow the upward trend to some extent. Towards the third week heavy turbulence of the sea with a strong current attended with bright sunshine and slight rise in temperature cause the phenomenon of "Animal exclusion" from the plankton. These conditions are most favourable for the creation of a diatom maxima and "zoo-plankton definitely avoids area where phytoplankton is thick" (Hardy, 1935). This diatom flowering is continued to the end of the month and is carried to the beginning of the next month.

*September*—The diatom flowering soon falls for a short period during which favourable conditions prevail for a slight increase in the activity of the Copepods. Again, simultaneous with the recurrence of fairly identical conditions as in August there is a repetition of the descent of the Copepod curve and an ascent of the diatom curve.

*October*—The Copepods are at their lowest ebb at the beginning of the month and consist of only a few specimens of *Acartia*, while the diatoms abound. Thenceforth, the variety and quantity of Copepods increase and *Temora*, *Paracalanus*, *Euterpina* and *Pseudocalanus* come into the planktonic picture. This condition together with the scarcity of diatoms continue for a short period, when due to the scarcity of diatoms the Copepods fall down.

*November*—From the beginning of November, there is a gradual ascent of the Copepoda with great enhanced activity of *Paracalanus*, *Acartia*, *Euterpina*, *Oithona* and *Pseudodiaptomus*. Diatoms show a weathering out and are very rare towards the end of the month.

*December*—At the beginning of December the Copepods are at their zenith of activity. The abundance of Copepods in December is similar to the peak of diatoms in August and September. December is a zoo-planktonic month.

*January and February*—In January there is an initial fall of Copepods which is soon made up and throughout the rest of the month and in February excepting the last week there is a steady production of Copepods.

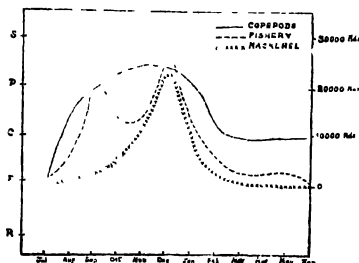
*March, April, May and June*—From March onwards there is only a very reduced activity of Copepods and they descend to very 'Rare' stage in May and June.

# VII. COPEPOD FLUCTUATION IN RELATION TO WEST COAST FISHERY FOR THE QUINQUENNium 1939-40 TO 1943-44

The fluctuations in the Copepod population in the West Hill plankton were correlated to those of the fishery of the plankton-feeding fishes of the Calicut Coast for the years 1939-40, 1940-41, 1941-42, 1942-43 and 1943-44.

## 1939-1940 (Fig. 5)

The Copepods begin to rise to a high level by September and continue to be in the peak period till December. The general fishery of the Coast

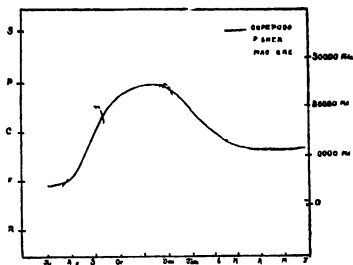


TEXT-FIG. 5. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1939-40.

also flourishes and reaches a peak in September, but falls down to a lower level in October, due to the prevailing North-East Monsoon, during which fishermen find it difficult to go out into the sea. Notwithstanding the temporary fall, the general fishery again rises to its maximum by December, from which month there is a steady fall, simulating the decline of the Copepod curve. As for the Mackerel fishery, the peak in September is absent but that in December coincides with both the general fishery and the Copepod peaks.

## 1940-1941 (Fig. 6)

The graph differs but slightly from the one just described (1939-40). The Copepods take longer time to establish their abundance and attain

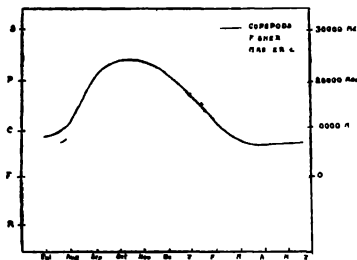


TEXT FIG. 6 Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1940-41

Plenty stage in October and maintain that level till January. The general fishery however rises to a peak in September a little ahead of the Copepodan abundance. The fall in general fishery due to the North East Monsoon is repeated this year as well. Nevertheless the general fishery soon regains its abundance and the highest peak is reached in December coinciding with the Copepodan maximum. The Mackerel fishery has only one peak as in the previous year which peak coincides with those of the Copepod and the general fishery. After December all three i.e. Copepod general fishery and Mackerel Fishery are on the decline.

#### 1941-1942 (Fig. 7)

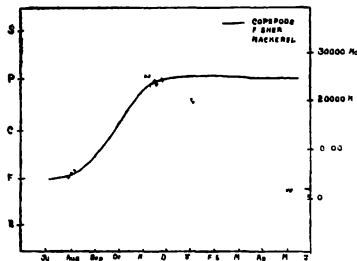
The Copepods begin to rise this year from August onwards and reach a very high peak by October from which month there is a gradual decrease in their abundance. The general fishery too closely following the Copepodan curve at first rises but later suffers a depression in September perhaps due to the strong North East Monsoon. The fishery takes a long time to recover and attains the second peak only in January by which time the Copepod curve is already on the descent. The Mackerel Fishery even though it exhibits slight activity in August and again in October reaches its maximum peak in January coinciding with those of Copepodan and the general fishery.



TEXT-Fig 7 Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1941-42

1942 1943 (Fig 8)

The Copepods take a longer time to rise up to the Plenty stage thus being achieved only in December. From this month the abundance of



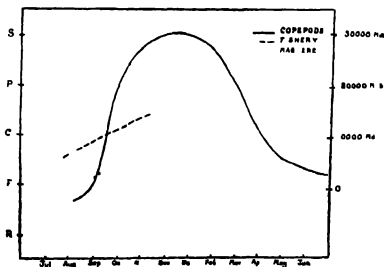
TEXT-Fig 8 Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1942-43

Copepods is maintained at almost the same level for the rest of the year. The general fishery curve runs almost parallel to the Copepod ascent and

the peaks in both are reached simultaneously, but from November onwards there is a slight fall. From this depression it seems as if the general fishery tried to emerge again, but soon after a slight rise descended to low levels unlike the steady Copepod curve. The Mackerel fishery curve runs almost parallel to the general fishery curve. The North-East Monsoon being less prolonged does not seem to have had any adverse effect on the fishery. It is not known why the fishery did not maintain a high level following the steady Copepod abundance.

1943-1944 (Fig. 9)

In September the Copepods increase in quantity and reach the Swarm stage in December. From February onwards there is a regular descent.



TEXT FIG. 9 Chart showing the correlation of Copepods with general fishery and Mackerel fishery of the West Hill Sea for the year 1943-44

of the Copepod curve. The general fishery is fair from the beginning of the year and its rise is gradual until the zenith is reached in January. From February onwards, there is a descent similar to the Copepod curve. The Mackerel fishery differs but little from the general fishery, the only noticeable difference being its low start at the beginning of the year. The Mackerel fishery also reaches its maximum in January and shows a decline from February onwards corresponding to the general fishery and Copepods. The absence of a depression in the fisheries during the North East Monsoon was due to the comparatively weak Monsoon which prevailed this year.

## VIII. COPEPODS AND FISHERIES

During the months of July and August, due to the Monsoon, there is very little fishery and the Copepods too are at their ebb. By September, the Copepods are on the ascent and the plankton-feeders too increase. Due to the North-East Monsoon, there is a fall in the fishery in the months of October and November. It is remarkable that the peak periods for both the Copepods and the Fishery are reached in December for all the five years. This condition extends to January also for two years, *i.e.*, 1942-43 and 1943-44 (Figs. 7 and 8). From February onwards there is a sudden fall in both the fishery and Copepods, probably due to upwelling of the coastal waters. From March onwards up to June, there is neither good fishery, nor do the Copepods rise above the 'Common' stage. In the year 1942-43, there was a remarkable abundance of Copepods. They ascended to the 'Plenty' stage in the month of October and continued in that stage for the rest of the year, and naturally the best fishery in all the five years under review occurred then.

## IX COPEPODS AND MACKEREL FISHERY

The Mackerel is the most important plankton-feeding fish. It is noteworthy that in all the five years the Mackerel fishery presents unimodal curves closely resembling the unimodal copepodan curves, with the peaks of the two coinciding while the general fishery shows bimodal curves (Figs. 4 to 8).

## X. DISCUSSION

Chidambaram and Menon (1946) have described in detail the seasonal occurrence of diatoms in the sea off Calicut and their relationship to the physical factors in the sea and weather conditions. The present investigations not only confirm their observations, but tracing the food-chain of the sea further show how the Copepods link diatoms to the fishery. The physical factors have little direct effect on Copepods but they influence the fluctuations of diatoms, which, in their turn cause Copepodal fluctuations. Hence it is that the two monsoons with their freshets of nutrient salts, the slight increase in temperature and salinity, increase in sunlight and calm waters, all seem to have a beneficent effect, though not immediately, on the Copepodal growth.

The correlation between diatoms and Copepods in the plankton is obvious. Earlier workers (Johnstone, 1911; Fish, 1925; Bigelow, 1926) stated that the "main copepodan population appears in times and places where prominent diatom flowerings were absent". But the Monsoons

caused a bloom setting "a nursery for young Copepods" (Wimpenny, 1926). However, these developing Copepoda grazed on the diatom patches "eating holes into it". So Copepods can be considered as a major factor in regulating the diatom population (Harvey, Cooper, Lebour and Russel, 1935).

The relationship between Copepodal fluctuation and the fishery is also apparent. The fishery of plankton feeding fishes in general and Mackerel in particular, coincides with the Copepodal fluctuations.

## XI ACKNOWLEDGEMENTS

This report has been compiled with the help of observations recorded at the West Hill Biological Station for the past one decade. We wish to express our indebtedness to the various research workers who recorded this data, as well as to Professor Ben Charan Mahendra for his constructive criticism and valuable suggestions in the preparation of the manuscript.

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## APPENDIX

## FISHES OF THE WEST HILL AREA FOUND TO FEED ON COPEPODS

<b>Family Clupidae</b>	<i>Sardinella fimbriata</i> (Cuv and Val) <i>Sardinella longiceps</i> (Cuv and Val) <i>Sardinella albella</i> (Val) <i>Sardinella jussieu</i> (Lee) <i>Macrura kelee</i> (Cuv) <i>Macrura ilisha</i> (Buch and Ham) <i>Opisthopterus tardoore</i> (Swainson) <i>Kowala coval</i> (Cuv)
<b>Family Engrulidae</b>	<i>Thrissocles mystax</i> (Schn) <i>Thrissocles dussumieri</i> (Val) <i>Thrissocles malabaricus</i> (Bloch) <i>Thrissocles kammalensis</i> (Blkr) <i>Anchoviella tri</i> (Blkr) <i>Anchoviella heteroloba</i> (Ruppel) <i>Anchoviella zollingeri</i> (Blkr)
<b>Family Dorosoridae</b>	<i>Anadontostoma chacunda</i> (Buch and Ham)
<b>Family Chanidae</b>	<i>Chanos chanos</i> (Forsk)
<b>Family Aridae</b>	<i>Arus dussumieri</i> (Cuv and Val)
<b>Family Hemiramphidae</b>	<i>Hemiramphus georgii</i> (Cuv and Val)
<b>Family Cynoglossidae</b>	<i>Cynoglossus semifasciatus</i> (Day) <i>Cynoglossus brachyrhynchus</i> (Blkr)
<b>Family Mugilidae</b>	<i>Mugil parsia</i> (Buch) <i>Mugil waigiensis</i> (Q G)
<b>Family Sciaenidae</b>	<i>Johnius carutta</i> (Block)
<b>Family Scombridae</b>	<i>Rastrelliger kanagurta</i> (Rupp)
<b>Family Carangidae</b>	<i>Caranx crumenophthalmus</i> (Blrk) <i>Caranx kurra</i> (Cuv and Val) <i>Caranx kalla</i> (Cuv and Val)
<b>Family Serranidae</b>	<i>Serranus fasciatus</i> (Forsk)
<b>Family Polynemidae</b>	<i>Polynemus sextarius</i> (Blkr)
<b>Family Lactariidae</b>	<i>Lactarius lactarius</i> (Schn)
<b>Family Leognathidae</b>	<i>Leognathus bindus</i> (Cuv and Val) <i>Leognathus splendens</i> (Blkr) <i>Leognathus insidiator</i> (Blkr) <i>Leognathus ruconius</i> (Ham) <i>Leognathus edentulus</i> (Blkr)

# SOME STAGES IN THE DEVELOPMENT OF THE PINEAL COMPLEX OF *CALOTES VERSICOLOR* (DAUD.)

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Received March 18, 1947

(Communicated by Prof. M. A. Moghe, F.A.S.)

## INTRODUCTION

SINCE Leydig's<sup>1</sup> pioneer paper dealing with the Parietal Organ in *Lacerta* and in *Anguis* a large number of papers on the structure and development of this organ in a series of vertebrates have been published by various authors. Baldwin Spencer (1887) in his excellent monograph on the "presence and structure of the Pineal eye in Lacertilia" gives a brief description of the structure of Pineal eye in some species of *Calotes*. It seems there is no other work dealing with the Pineal organ of *Calotes* although Dendy (1899 a), (1899 b), (1907), (1911), Nowikoff (1910), Boveri (1925), and others have described the structure and development of pineal organ in many reptiles. Gladstone and Wakeley (1940) give a summary of all the work done on this organ from morphological, histological, cytological, embryological and medical points of view.

The present paper attempts to give a brief description of some developmental stages of pineal organ in a series of *Calotes* embryos. This study was undertaken while examining a series of sections of the embryos of *Calotes versicolor* (Daud.) for the purpose of observing the development of some of the chondrocranial elements. Unfortunately very young stages of embryos were not present in my collection.

My study confirms, except in some details, the observations made by previous workers. Whereas the general plan of the development of the pineal organ in *Calotes* in no way differs from that of *Sphenodon* and *Lacerta*, the structure of the Paraphysis in this case is simpler and the lens arises at a comparatively later stage.

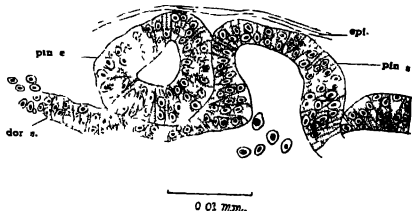
## MATERIAL AND METHODS

The material for this paper was collected in Nagpur during July to September 1944, and was fixed in Bouin's fluid, sections of the heads of embryos were stained in Hæmatoxylin and counterstained in Eosin.

<sup>1</sup> *Die Arten der Saurier*, 1872, p. 72

## DESCRIPTION OF STAGES

The earliest stage in my collection (head length 3.5 mm.) shows the two Pineal vesicles lying one behind the other over the roof of the forebrain (Text-Fig. 1). Of these vesicles, the anterior (*pin.e.*) is completely closed on all sides and is situated slightly towards the right side of the median



TEXT-FIG. 1—Sagittal section through the head of a young embryo of *Calotes versicolor* (Daud). *dor.s.*, dorsal sac, *epi.*, epidermis, *pin.e.*, pineal eye, *pin.s.*, pinea sac  $\times 3305$ .

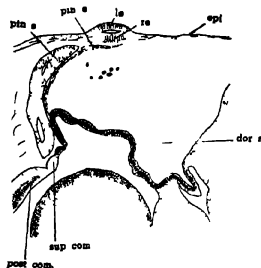
sagittal plane. It is oval in shape with the upper surface slightly flattened. It is covered above by the epidermis (*epi.*) of the head, and behind it lies the posterior vesicle (*pin.s.*) and below it, the wall of the Dorsal Sac (*dor.s.*). This vesicle will ultimately develop into the Pineal eye

The posterior vesicle (*pin.s.*) is a finger-shaped structure arising from the forebrain. It is hollow and is in communication with the cavity of the forebrain. In front of it is the anterior vesicle (*pin.e.*) with which it does not communicate. Subsequently it will give rise to the Pineal Sac. The wall of the brain just in front of the Posterior Vesicle and below the anterior one will form the Dorsal Sac.

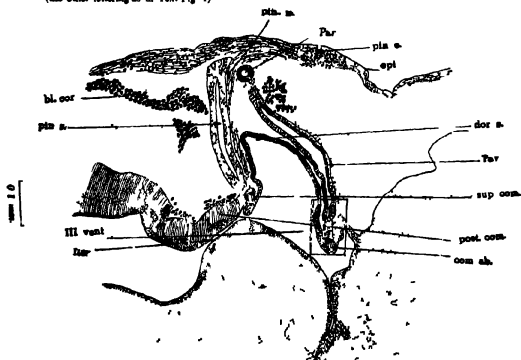
Histologically, both these vesicles resemble the wall of the brain from which they arise.

In the next stage (head length 4 mm.) (Text-Figs. 2 and 3), the pineal complex is more fully developed, and the pineal eye appears to be fully formed (*pin.e.*), its upper portion developing into a lens (*le.*) and the lower portion into retina.

The mesoblastic tissue between the brain roof and the epidermis separates the pineal eye from the forebrain and the pineal eye has apparently



TEXT FIG 2—Median sagittal section through the head of an embryo of *Calotes versicolor* (Daud) of approximately 4 mm head length in the region of the pineal eye *le* lens *post com* posterior commissure *re* retina of the pineal eye *sup com* superior commissure (the other lettering as in Text Fig 1)

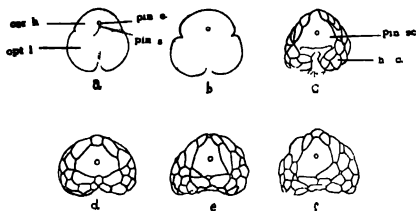


TEXT-FIG 3 Median sagittal section through the head of an embryo of *Calotes versicolor* (Daud) of 4 mm head length. The section is slightly oblique and does not pass through the middle of the pineal eye *bl cor* blood corpuscle *com ab* commisura aberrans *par* paraphysis *III vent*, third ventricle (other lettering as in Text Figs 1 and 2)

lost all connections with it. The pineal sac (*pin s*) develops as a glove finger shaped structure bent anteriorly and ending well behind the pineal eye. From the posterior end of the pineal eye the pineal nerve (*pin n*) goes back below the pineal sac anterior to it and enters the superior commissure (*sup com*) where it enters Habenular ganglion. The paraphysis (Text Fig 3 *Par*) also appears to arise from the forebrain as a hollow finger shaped outgrowth at the posterior extremity of the Dorsal Sac (*dor s*). The limit of the Dorsal Sac is defined anteriorly by the paraphysis and posteriorly by the Pineal sac.

From this stage onwards all these organs persist as such and show the same structure. In later stages the pineal eye undergoes certain changes in its shape and in the amount of pigment present in it. Other parts do not show any marked change.

While these organs are being formed internally the pineal eye is seen externally from 3.5 mm head length onwards as a circular dark blue spot (Text Fig 4 *a* to *f pin e*) situated nearly in the middle of the head in the



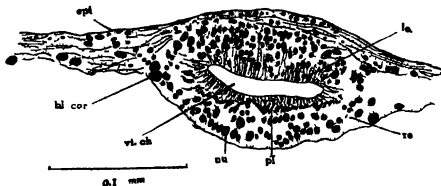
TEXT FIG 4.—Dorsal view of the developing embryos of *Calotes* showing developing pineal scale over the pineal eye. *cer h* cerebral hemispheres, *h sc* scales of the head, *opt l* optic lobes, *pin e* pineal eye, *pin s* pineal sac.

space bounded in front by the cerebral hemispheres (*cer h*) and behind by the optic vesicle (*opt l*). Immediately behind the pineal eye another blue spot considerably smaller than the pineal eye marks the position of the anterior end of the pineal sac (*pin s*). Very soon scales develop in the head region (*sc*). Above the pineal eye a large transparent median scale, polygonal in shape and completely free from pigment, makes its appearance (*pin sc*). This pineal scale persists as such in the adult.

# DISCUSSION

**The Pineal Eye**—The earliest appearance of the pineal eye is shown in Text-Fig 1. It comprises of the anterior vesicle (*pin e*). No other differentiation in the wall of the vesicle such as into an upper lens and a lower retina is visible although the vesicle is completely separated both from the posterior vesicle and the brain roof. The differentiation of the anterior vesicle into lens and retina therefore appears to occur at a later stage. *Calotes*, in this respect, differs from *Lacerta* (Nowikoff 1910), and *Sphenodon* (Dendy, 1911), in both of which the upper wall of the anterior vesicle thickens quite early sometimes long before the two vesicles are constricted off from the brain and from each other.<sup>1</sup> A comparison of Text-Fig 1 with Figs 30, 31, Plate 5 of Dendy 1911 and Fig 6 Plate 3 of Nowikoff, 1910, clearly shows the difference. Both in Nowikoff's and Dendy's figures the upper wall of the anterior vesicles has thickened to form the lens, but in *Calotes* embryos of roughly the same developmental stage no signs of differentiation are visible as yet in the anterior vesicle.

There is a considerable gap between the first stage (head length 3.5 mm) and the second stage (head length 4 mm), the latter represents a more advanced condition (Text-Fig 5).



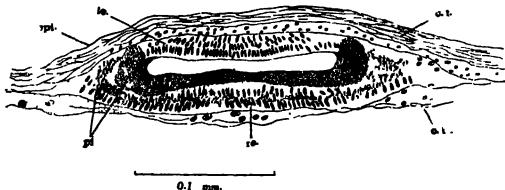
TEXT-FIG 5.—Transverse section through the pineal eye of a *Calotes* embryo of 4 mm head length. *nu*, nucleus, *pl* pigment, *vl ch*, vitreous chamber.

The lens is formed from the upper portion of the wall of the anterior pineal vesicle (Text-Fig 1, *pin e*) the region which is in direct contact with the superficial epiblast of the head. The cells of the pineal eye in this region

<sup>1</sup> "The differentiation of the wall of the optic vesicle into lens and retina takes place at a remarkably early stage of development. It may commence even before the two pineal outgrowths have separated from one another which occurs at stage 0 (fig. 30)."—Dendy *Phil Trans Roy Soc London*, 1911, 201, 265.

become elongated and consequently the wall is thick (Text-Fig. 5, *le.*). At this stage the area of the lens in proportion to that of the retina is very small, the wall of the lens is thickest in the middle but gets thinner as it approaches the retinal region. The lens thus has a biconvex shape at this stage. The cells of the lens are lenticular and they contain elongated fusiform nuclei. No pigment was observed in the lens at any of the stages. There is no sharp constriction at the junction of the lens with the retina and the lens can be distinguished from the retina by its elongated cells and absence of pigment in it. Between the epidermis of the head (Text-Fig. 5, *epi.*), and lens there is a clear and transparent mass of connective tissue (*ct.*). In later stages when the pineal eye becomes dorsoventrally compressed and consequently flattened the biconvex structure of the lens becomes less marked and the area of the lens also increases.

The retina of the pineal eye consists of the usual elements found in other Lacertileans. In 4 mm head length embryo the wall of the retina adjoining the vitreous chamber is almost free from nuclei (Text-Fig. 5). This area is packed with dark brown pigment granules (*pi.*). The outer region of the retina is limited by a basal layer of cells with nuclei roughly arranged in a single row (*nu.*). Between the outer basal layer and the inner pigmented region are numerous irregularly scattered nuclei belonging to pigment cells, the ganglion cells, and other elements of the retina. The vitreous chamber (*vi. ch*) which is quite conspicuous does not appear to contain any structures in it such as are found in *Sphenodon* and *Lacerta*. In the 6 mm. head length stage (Text-Fig. 6), the pineal eye appears more



TEXT-FIG. 6. Transverse section through the pineal eye of a *Calotes* embryo at 6 mm. head length. *c.t.*, connective tissue; *c.t.f.*, connective tissue fibres.

compressed dorsoventrally and the vitreous chamber becomes narrower and more elongated. The amount of pigment in the retina increases

### *Stages in Development of Pineal Complex of C. versicolor (Daud.) 201*

considerably (Text-Fig 6, *pi*) and a thick mass of pigment occupies nearly half of the region of the retina adjoining the vitreous chamber. The nuclei of the retina are more regularly arranged. Besides the inner compact mass of pigment, scattered pigment granules also occur throughout the retina (*pi*).

**Pineal sac**—The posterior vesicle (Text-Fig 1, *pin s*) already described, develops into the pineal sac. Beginning as a hollow finger-shaped outgrowth arising from, and in communication with, the cavity of the forebrain the pineal sac becomes progressively elongated as the epidermis of the head separates from the roof of the forebrain owing to the development of the mesoblastic tissue (Text-Figs 2 and 3). The pineal sac from 4 mm head length stage onwards retains a uniform structure. It originates towards the posterior end of the dorsal sac, runs upwards and then onwards and ends slightly behind the pineal eye. In very early stages the anterior end of the pineal sac can be seen as a tiny blue spot just behind the pineal eye in the dorsal groove of the head (Text-Fig 4 *a, b, c*, etc). In advanced stages, however, the pineal sac becomes more deeply situated and hence cannot be seen externally. Originally the cavity of the pineal sac is continuous with the third vesicle (Text-Fig 1), but this connection is obliterated when the superior commissure (Text-Figs 2 and 3, *sup com*), and posterior commissure (*post com*) come together and abut against each other. The original place where the cavity of the pineal sac opened into the cavity of the brain is clearly seen in advanced stages as a groove between the posterior and superior commissures (Text Fig 4). The connection of the pineal sac with the brain-roof is however retained through the proximal solid end of the pineal sac. The pineal sac is a hollow structure although its cavity appears to be obliterated in the bend.

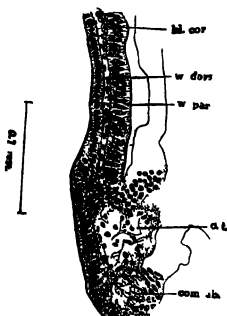
The pineal sac is a hollow structure although its cavity appears to be obliterated in the bend. It is a multicellular thick-walled structure. The cells are more or less irregularly arranged. In general, its structure resembles that of the retina of the pineal eye. In many embryos, presence of pigment was observed in the distal extremity of the pineal sac. Dendy (1899 *b*) has recorded the presence of pigment near the distal extremity of the pineal sac in an embryo of *Sphenodon*. Dendy (1911) also described the presence of pigment in the distal extremity in adult *Sphenodon*.

**The Pineal Nerve**—In the earliest stage, the pineal nerve is not seen. At 4 mm head length the pineal nerve is quite distinct (Text-Figs 2 and 3, *pin n*). It enters the pineal eye towards the posterior region and not in the middle. Traced backwards from the pineal eye the nerve runs at first

nearly parallel to the brain and it bends near the distal extremity of the pineal sac and runs for the rest of its course in close connection with the pineal sac anterior to it. The pineal nerve is seen finally to enter the superior commissure beyond which its fate could not be traced.

*The Dorsal Sac*—The roof of the forebrain just above the optic thalamus forms the Dorsal Sac (Text Figs 2 and 3 *dors*) Posteriorly the sac ends near the base of the pineal sac in the superior commissure (Text Figs 2 and 3 *sup com*) and its anterior limit is marked by commissure aberrans (*com ab*). The dorsal sac is a broad based triangle in communication with the third ventricle. It is covered anteriorly by the paraphysis (Text Figs 2 and 3, *Par*) and posteriorly by the pineal sac (Text Figs 2 and 3, *pin s*).

The Dorsal Sac is a thin walled structure consisting of a single row of cubical cells with small more or less spherical nuclei (Text Fig 7 *w dors*).



TEXT FIG 7—A magnified figure of the region enclosed within the rectangle in Text Fig 3 to show the structure of the wall of the paraphysis and the dorsal sac *w dors* wall of dorsal sac *w par* wall of the paraphysis.

The nuclei are regularly arranged and placed towards the inner side of the wall.

*Paraphysis*—The paraphysis occupies the same position in the embryos of *Calotes* as described in the embryos of *Sphenodon* and *Lacerta*, but its

structure in *Calotes* embryo is less complicated. It develops as a thin finger-shaped process just in front of the dorsal sac (Text Fig 3, *Par*) and communicates with its cavity through the third ventricle. It bends back over the wall of the dorsal sac covering it for the greater part of the length. It runs close and parallel to the pineal sac for a short distance the space between the two being filled with mesoblastic tissue, blood vessels and nerve fibres.

The paraphysis in *Calotes* embryo differs from that in *Sphenodon* (1899 b, 1911), and *Lacerta* (Nowikoff, 1910) in the absence of folds and tubules in its walls. Its blood supply also does not appear to be so abundant. The histology of the paraphysis is almost identical with that of the dorsal sac. The wall of the paraphysis is, however, thicker than that of the Dorsal Sac and its nuclei are more elongated and less regularly arranged (Text-Fig 7, *w par*). It does not show the syncytial structure with the nuclei arranged in regular rows as in *Sphenodon* (Dendy, 1910). In *Calotes* embryo the paraphysis has well-defined outlines. As stated above it further differs from other Reptilian embryos in not being a complex tubular structure richly supplied with blood vessels. The paraphysis in almost all *Calotes* embryos examined by me is a simple structure and is neither produced into a convoluted tube nor it is very highly vascular.

#### ACKNOWLEDGEMENT

This work was done in the Zoology Laboratory of the College of Science as a part of a larger investigation on the development of the pineal organ in the Vertebrates. I am grateful to Prof M. A. Moghe for valuable guidance.

#### SUMMARY

- 1 The various parts of the pineal complex, viz, the pineal eye, pineal sac, pineal nerve, dorsal sac, and paraphysis develop in the same way as in *Sphenodon* and *Lacerta*.
- 2 The lens of the pineal eye of *Calotes versicolor* appears at a later stage of development than in *Sphenodon* and *Lacerta*.
- 3 The paraphysis in the embryos of *Calotes versicolor* is a simple structure, neither produced into convoluted tubules nor richly supplied with blood vessels.

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# THE EFFECT OF THE INTERACTION BETWEEN IONS, DRUGS AND ELECTRICAL STIMULATION, AS INDICATED BY THE CONTRACTION OF HUMAN UNSTRIATED MUSCLE

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Received April 25 1947

The experiments performed on *Mytilus*, frog, mammalian and avian unstriated muscle (Singh, 1938a 1939, 1940, Singh, Singh and Muthana, 1947) have been performed upon human unstriated muscle for purposes of comparison

## EXPERIMENTAL

The human appendix has been used. The method of stimulation was as described previously (Singh, 1938a, 1940). The appendices were removed by one of us (A K M Khan) during abdominal operations at the civil hospital. Immediately after operation the condition of the appendix as regards its inflammatory condition was noted. In the beginning, a histological study of the sections was undertaken, but later on this was abandoned, as no correlation was found between the response and the histological findings. Further no correlation was found between the condition of the appendix as noted externally, and the response as our experiments were of a qualitative nature, thus the condition of the appendix was not of any significance in these experiments.

## RESULTS

The responses of the human appendix show some fundamental differences from those of unstriated muscle from animals hitherto used.

*Effect of temperature*—The optimum temperature in six appendices, two normal and four inflamed, for the response to alternating current, was higher than in the dog's stomach, dog's retractor penis, fowl's gut, or rabbit's gut, it was 37–40° C (Fig 1). It appears that human muscle acts best when at the body temperature, and a little above which may be encountered in fever, human muscle has therefore, a more restricted range for normal activity. It is possible that for normal functioning, the range of variation allowed in the environmental factors, becomes more restricted with ascent

in the scale of evolution; in other words a more perfect homeostasis is demanded

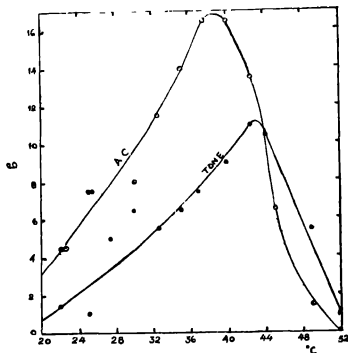


FIG. 1. Appendix. Effect of temperature on the response to alternating current (8 volts for 10 seconds every 15 minutes) and tone.

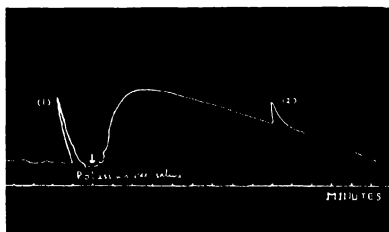


FIG. 2. Appendix. Contraction in the absence of potassium. Contractions (1) and (2) by alternating current (8 volts for 10 seconds). Potassium free saline is added at the arrow. Note the contraction.

### *Effect of Interaction between Ions, Drugs & Electrical Stimulation 207*

The optimum temperature for the response to potassium is the same as that for alternating current; this differs from results on other muscles, in which the optimum temperature for potassium is less than that for alternating current and so less than 37° C.

**Other differences.**—Human muscle contracts in the absence of potassium (Fig. 2). Anions, Br, I, NO<sub>3</sub>, SCN, and drugs eserine, acetylcholine, in small concentrations cause contraction, which is not antagonistic to alternating current (Fig. 3). These results have occasionally been obtained on other muscles. Higher concentration of the above substances may be antagonistic to alternating current.

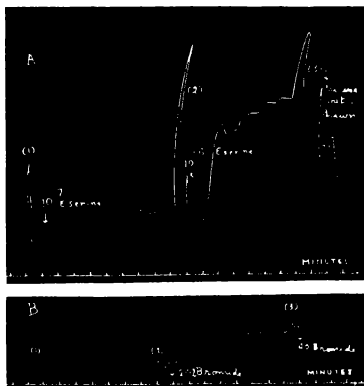


FIG. 3. Appendix. A. Contraction (1) is constant response to alternating current in saline.  $10^{-7}$  eserine sulphate added at first arrow; increase in tone as well as the response to alternating current (2). Addition of  $10^{-6}$  eserine sulphate at second arrow greatly increases tone, but the response to current decreases (3); eserine is then withdrawn and the muscle relaxes at third arrow. B. First two responses to alternating current in saline. 20 per cent. of chloride is then replaced with bromide; tone increases as well as the response to current (3).

*Effect of ammonium*—Ammonium initially produces an inhibition to which the muscle adapts. Increase in the concentration of ammonium produces further inhibition and adaptation. This can be repeated till adaptation to inhibition ceases and the muscle finally relaxes (Fig 4). These

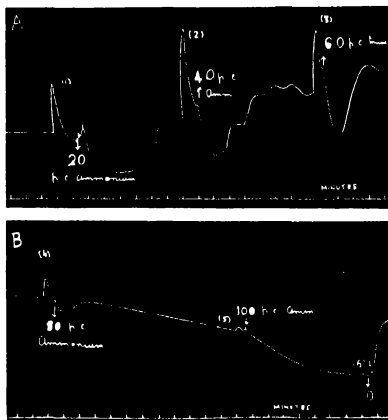


FIG 4 Appendix (1) (2) (3) (4) (5) (6) contractions produced by alternating current. 20 per cent of the sodium of the saline replaced by ammonium after (1) note inhibition from which the muscle recovers. Similarly increasing concentration of ammonium added after each contraction produces inhibition from which the muscle recovers. With 80 to 100 per cent replacement adaptation diminishes and inhibition becomes permanent.

results resemble exactly those produced on *Mytilus* muscle with contraction by alternating current (Fig 5) (Singh, 1938b). This suggests some intimate relationship between inhibition and excitation (Singh, 1945).

The optimum pH for the response to alternating current is 8 and 7

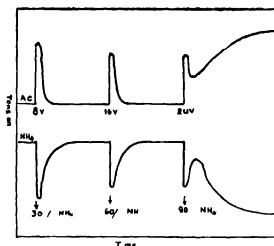


FIG 5 Diagrammatic comparison of contraction in *Mytilus* muscle and inhibition in the appendix. In the upper curve (*Mytilus* muscle) the voltage of alternating current is increased in steps and in the lower curve (Appendix) the concentration of ammonium is similarly increased

#### DISCUSSION

Human muscle thus differs from muscle of lower animals in many important respects. The fact that tone, the response to potassium and that to alternating current may be affected similarly, suggests that in human muscle, adaptation plays the dominant role. This also throws light on the mode of excitation by a substance.

When the muscle is stimulated, the tension produced subsides owing to adaptation. If the excitatory process be termed as V, and adaptation as U, then the tension developed is a function of (V-U). Now, (V-U) can increase in two ways, first, by increase of V, and secondly, by decrease of U. It follows therefore, that excitation may take place by lowering of threshold value of adaptation, the response to potassium and alternating current as well as tone would then be identically affected. If adaptation is due to release of calcium, then excitation would be produced by suppression of ionised calcium in the membranes or elsewhere. Thus increase in calcium would produce inhibition, and decrease, excitation. This is probably the mechanism of surface action by various drugs and ions. The other way of producing excitation is by producing a difference in ionic concentration on the two sides of the muscle membrane.

## SUMMARY

Human unstriated muscle differs from unstriated muscle of lower animals in the following respects

(1) The optimum temperature for excitability is higher, 37°

(2) Many substances affect the tone as well as the excitability to alternating current and potassium similarly. It is suggested that this is due to decrease of adaptation, as an increase produces inhibition

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## THE ACTION OF DIRECT CURRENT ON UNSTRIATED MUSCLE

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WINTON (1937) found that the stimulation of *Mytilus* unstriated muscle by direct current results in slow relaxation after contraction. He did not find any effect of polarity of the current on the mechanical response. Singh (1938 b) found that this tonic contraction or slow relaxation was due to the action of ions in the saline, as it was produced when stimulating ions were present in the saline and was antagonised by agencies that opposed the action of these ions. In striated muscle the mechanical response during fatigue is affected by polarity of direct current. Heilbrunn (1937). Singh (1937) showed that though *Mytilus* muscle may become inexcitable to all forms of stimulation, when the chloride of the saline is replaced with cyanide, it still responds to cessation of direct current.

In the present research, the effects of direct current, which differ in many respects from those of alternating current, were elucidated.

### EXPERIMENTAL

The muscle used was that from the frog's stomach, circular strips were used. They were stimulated with direct current by two methods. (1) the first method was that described previously (Singh, 1938 a). (2) In the second method the muscle was stimulated by either the anode or the cathode varying from 1.4 to 1.5 volts, using Zn ZnSO<sub>4</sub> non-polarisable electrodes, the indifferent electrode was on one end of the muscle which was killed by heat.

### RESULTS

When the frog's muscle is stimulated with direct current, it produces three contractions, one while the current is flowing, the other when the current stops after a latent period of about 2 to 10 seconds, and a third a few seconds (10-60), after the cessation of the current (Fig. 1).

*Relation between make and break contractions*—The make and break contractions may be affected identically or oppositely. When they are affected oppositely, their magnitude bears an inverse ratio, so that the total tension may approximately remain constant (Fig. 2). Thus substances that

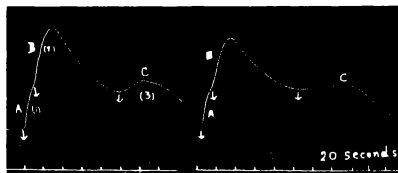


FIG 1 Frog's stomach muscle Contraction by 14 volts direct current (D.C.) for 10 seconds A the make contraction between first two arrows B the break contraction and C the third contraction which is usually brought out by eserine (1 in  $10^4$ )

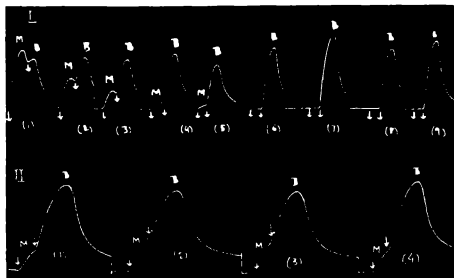


FIG 2 Frog's stomach muscle Stimulation by constant current 14 volts for 10 seconds every 10 minutes Upper curves 1st 5 contractions in saline 6th and 8th contractions in 100 per cent  $\text{NH}_4\text{Cl}$  replaced with  $\text{NH}_4\text{Cl}$  6th and 8th contractions in 100 per cent  $\text{NH}_4\text{Cl}$  7th and 9th contractions with the current reversed [note absence of make contraction (M = make and B = break contraction.) Lower curves 1st and 4th contractions with the current in the same direction 2nd and 3rd contractions with the current in the opposite direction the make contraction becomes larger and the break smaller

increase the make contraction, under such circumstances, will correspondingly decrease the break contraction. With great increase in the make contraction the break contraction may be almost abolished (Fig 3). The properties of the make contraction are those of the contraction produced by alternating current and those of the break contraction are similar to those

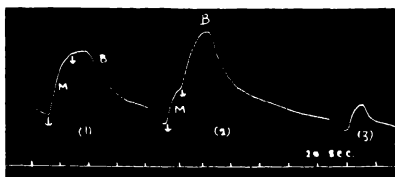


FIG 3 Frog's stomach muscle. Contraction by 14 volts D C 10 sec. First contraction in acetylcholine ( $1 \text{ in } 10^4$ ) the break contraction is very small. The second contraction with the current reversed. The third contraction is in adrenaline ( $1 \text{ in } 10^4$ )

of the potassium contraction (Singh, 1938 *a*). If the make contraction decreases, then the break contraction may increase, so that as the former vanishes, the latter alone remains. Sometimes both the contractions are absent and only the third contraction is obtained. This produces a curious phenomenon in that the muscle remains quiescent during the passage of the current, but contracts after the lapse of a few seconds or minutes. Such a contraction is best observed if spontaneous contractions are absent. The contraction is antagonistic to that produced by alternating current. It is akin to the secondary contraction (Singh, 1938 *a*), or is possibly due to nerves (Singh and Singh, 1947). This reminds one of the long latent periods of gastric or pancreatic secretion to vagus stimulation.

**Effect of stimulation**—During the beneficial effect of contraction, the make contraction increases, and the break contraction decreases. To begin with, the muscle may be inexcitable to make but may give a large response to break of the current. During fatigue the make contraction decreases, and the break contraction increases. During both these phases, these contractions may be affected indifferently. This shows that during fatigue and the beneficial effect of contraction, factors arise which affect the two excitabilities in the same and in the opposite direction respectively.

**Effect of strength of stimulus**—With some increase in voltage, at first, both the contractions increase, but thereafter the make contraction increases at the expense of the break, so that with high voltages (40–50 volts D C), the break contraction may be abolished (Fig 4). The latter contraction may, however, reappear, if the response now begins to decline with increase in voltage. The above observations suggest that some common factor which is probably ionic, is responsible for both the contractions, so that if it is

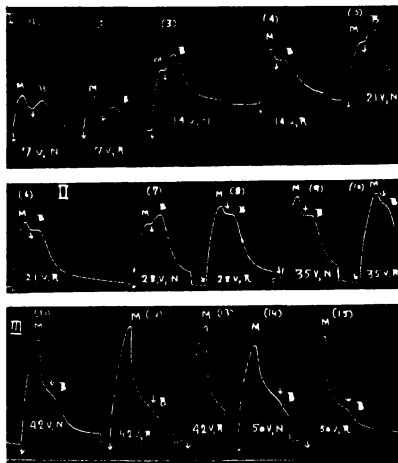


FIG. 4. Frog's stomach muscle. Contraction by D.C. M = make contraction, B = break contraction. N = normal direction, R = reversed direction. Note that in the lowest figure, in the thirteenth contraction with 42 volts, the break contraction has been abolished.

used more for the make contraction, less of it remains for the break contraction.

**Effect of temperature**—The optimum temperature for the make contraction is 25 to 30° C and that for the break contraction 15 to 20° C. Thus the break contraction belongs to the potassium group like the alternating current off-contraction.

**Effect of ions and drugs**—The effect of following substances was tested: (1) Monovalent cations, Li, Na, NH<sub>4</sub>, K, H. (2) Divalent cations, Ca, Sr, Ba, Mg. (3) Monovalent anions, Br, NO<sub>3</sub>, I, SCN, CN. (4) Drugs, adrenaline, acetylcholine and eserine. The effect of these substances on the

make contraction resembles that on the contraction produced by alternating current, and the effect on the break contraction, that on the potassium contraction. The effects of ammonium and potassium are interesting. If all the sodium of the saline is replaced with ammonium or if 20-40 per cent is replaced with potassium, then the make contraction disappears and only the break contraction remains, suggesting that the latter is dependent upon ions in the saline. All of the sodium may be replaced with potassium, and the muscle still responds to the break of the current (Fig. 5). This suggests

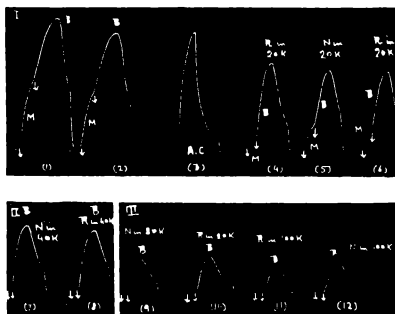


FIG. 5. Frog's stomach muscle. Stimulation by D.C. 14 V/10 sec. I. First two contractions with curves in normal and reversed direction, 3rd contraction by alternating current 8 V/10 sec for comparison. 4th, 5th and 6th contractions in saline with 20 per cent of sodium replaced by potassium (K in reversed direction, and N in normal direction). II and III are self-explanatory. K = percentage of potassium replacing sodium of the saline. In curves 7 to 12 the make contraction is absent.

that the inexcitability in excess of ammonium and potassium is not due to damage but to some redistribution of ions in the muscle. Hydrogen ions too (pH 5) produce a similar action. Similarly all the sodium chloride of the saline can be replaced with calcium and strontium chlorides; barium produces similar effect if 20 per cent of sodium is replaced. Magnesium, on replacing all the sodium chloride, and adrenaline (1 in  $10^7$ - $10^8$ ) have opposite action; the make contraction remains but the break contraction disappears.

*Effect of polarity of current*—The magnitude of the total response and that of the individual make and break contractions, varies with the directions of the current. The effect on the make and the break contraction is reciprocal. If there is no appreciable difference between the responses when the current is reversed, it can be produced by altering the ionic content of the saline either by replacing the sodium or the chloride ion with some other ion, or altering the total concentration of ions by altering the sodium chloride content of the saline. Polarity may affect the response in the electrolyte-free medium.

*Effects of polar stimulation*—On make and break of the current, contractions occur both at the anode and the cathode: the anodal contraction at the make being smaller than the cathodal. The properties of the make contractions are similar to those of the contraction produced by alternating current, and those of the break contraction are similar to those of the potassium contraction. In excess of potassium, as the make contractions disappear, the break contraction becomes powerful, the anodal contraction being the biggest. If contracture is first induced in frog's muscle, or in the guinea pig's uterus, then inhibition is produced instead of contraction, the anodal inhibition at the make being greater than the cathodal. Thus either inhibition or contraction may occur at the cathode or the anode on make or break of the current, this is in agreement with recent work on nerve (see Wiggers, 1944).

#### DISCUSSION

The contraction produced on break of the direct current depends upon two factors: (1) Changes in the saline; (2) The make response. The make and the break contractions are affected reciprocally, so that if any substance is added to the saline the break contraction will be affected by change in the saline as well as by change in the make contraction. Hence the response is rather irregular.

The break contraction is similar to the alternating current off-contraction. It can be produced in the electrolyte-free medium, so the assumption that it is due to the leakage of ions from the fibres is justified. It is also increased by ions outside the muscle fibres, so that it is produced by leakage of ions into an outer zone (Singh, 1944).

The muscle may be inexcitable to all forms of stimulation except that due to break of a constant current, this suggests that one of the factors that causes diminution in excitability is some rearrangement of ions. This cannot be explained on the assumption that the interior of the muscle fibres is uniform, and that they are surrounded by only one membrane.

The fact that the contraction on make of the constant current, is antagonistic to that on break, is not explained by the current theories of excitation by constant current. The fact that the response varies with polarity, suggests that the membranes of the muscle are not equally permeable in both directions. This would produce rectification, and so account for the stimulating action of alternating current.

#### SUMMARY

(1) The properties of the contraction produced by break of a constant current are similar to those of the alternating current off contraction, the make contraction resembles that produced by alternating current.

(2) The muscle responds to break of a constant current when it may be inexcitable to all other forms of stimulation, it may respond when all the sodium chloride of the saline is replaced with chlorides of lithium, ammonium, potassium, calcium, magnesium and strontium or in acid solutions (pH 5).

(3) Magnesium and adrenaline abolish the break contraction.

(4) The response differs with polarity of the direct current, this suggests that the permeability of the membranes is different in the two directions. Stimulation by alternating current is probably due, therefore, to rectification.

(5) The make and the break contractions bear a reciprocal relation to each other.

(6) With polar stimulation, the results are very complicated, contraction or inhibition may occur at the anode or the cathode on make or break of the current.

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6 JAN 1948

## STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part I Reproduction and Breeding Seasons in the South Indian  
Vespertilionid Bat *Scotophilus wroughtoni* (Thomas)

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(Communicated by Prof M A Moghe F A Sc)

(With Two Plates)

### INTRODUCTION

THIS is the first of a series of papers on the embryology of Microchiroptera. It attempts to record the breeding seasons of one of the species of insectivorous bats—*Scotophilus wroughtoni* (Thomas), collected at a place about 18 miles from Bangalore (South India). The climatic conditions of this place do not vary much during the year, the place being in the tropical zone. The present paper does not attempt to describe in detail the histological changes which occur in the reproductive tract of the bat during breeding and non-breeding seasons. This will be dealt with in a subsequent paper. It attempts only to record the salient features of the cyclical changes as observed in different months of the year and other phenomena of special interest in relation to the breeding habits of the bat. The work was based entirely upon the collections of wild specimens since the taming of these bats in the laboratory was found to be impossible. Captivation and consequent domestication of these bats might have considerably impaired the normal sexual rhythm, and might thus have given misleading results.

### HISTORICAL

The subject of the reproductive process of bats has engaged the attention of many workers for over a century. A review of the literature dealing with the reproduction of the insectivorous bats has been made by several authors. One such attempt was made by Duval (1895 a) who reviewed all the earlier work. Later a good summary of the literature was given by Hartman (1933). Baker and Bird (1936) in a paper on the "Seasons in a Tropical Rain-forest (New-Hebrides) Part 4—Insectivorous bats" gave a short resumé of the work done on the reproductive cycle of the insectivorous bats. At the time when Baker and Bird published their paper they were

almost the pioneer workers on the tropical species of microchiroptera. Since then quite a large number of workers have described the reproductive process in bats, not only of the temperate and cold climates but of the tropics also. Particular mention must be made of the valuable work done by Harrison Matthews (1937) and Mary J. Guthrie (1933) on the European, South African, and American bats. Harrison Matthews' record of the breeding seasons of the South African bats, though based on a very imperfect collection, gives a fairly clear idea of the sexual rhythm of the tropical bats.

Pagenstecher (1859) was almost the first to notice that there was something peculiar about the breeding habits of the bats. Working on *Pipistrellus pipistrellus* in Germany, he noticed that in winter the uterus of the female was swollen, and this swelling was due to the presence of live spermatozoa, though there was no sign of a ripe Graafian follicle in the ovary. He naturally concluded that copulation occurred in the bat earlier than ovulation and the sperms were capable of being stored in the genital tract of the female for a fairly long time, throughout the winter.

Van Beneden (1875) also observed sperms in the uteri of bats, but concluded that fertilisation occurred immediately after copulation and the fertilised ovum remained dormant till the end of winter. Emier (1879 *a* and *b*), however, confirmed the view of Pagenstecher and showed that in *Pipistrellus pipistrellus* and *Nyctalus noctula* copulation occurs late in autumn and the sperms hibernate during winter inside the uterine tract of the female.

Benecke (1879) and Fries (1879), working on a number of species—*Pipistrellus pipistrellus*, *Plecotus auritus*, *Vespertilio murinus*, *Vespertilio nathusii*, *Rhinolophus hipposiderus*, came to the conclusion that copulation occurred before hibernation and the sperms lived in the uterus of the female throughout the winter and ovulation and fertilisation took place during early spring. The young born in the summer do not copulate in the same season. They thus believed that spermatozoa hibernated in the uterus of the female for a period of at least 4½ months.

Later, Rollinat and Trouessart (1895-97) published their classical work dealing with the reproduction of two different species of microchiroptera, *Vespertilio murinus* and *Rhinolophus ferrum-equinum*. They clearly stated that sperms existed in a dormant state throughout the winter for about 4½ months, from October to the beginning of April, and ovulation and fertilisation occurred at the beginning of spring 8-10 days after the bats 'awoke' from their winter hibernation. Further their experiments with hibernating bats indicated that if the 'sleeping' females were brought to a warm room, ovulation, and consequent fertilisation and pregnancy resulted.

Grosser (1903) described a very remarkable phenomenon occurring in *Nyctalus noctula*, wherein copulation occurred very early—in July or August—and at the end of autumn the cervical canal was blocked by an increase in the amount of connective tissue and the sperms were thus stored up in the vagina. Ovulation and fertilisation occurred at the end of March or in early April when the genital canal was found to be free. This phenomenon which was not described in any other species proved beyond any doubt that copulation must have occurred early in winter or late autumn and fertilisation and pregnancy during spring and summer. Such a blocking of the vaginal passage was not noticed in *Vespertilio murinus* or *Placotus auritus*.

A parallel instance has been recorded by Courrier (1927) in the males of *Pipistrellus pipistrellus* where he noticed that the testes had degenerated at the beginning of winter leaving only the spermatogonia and Sertoli cells, and the testes resumed activity only in the next autumn. He had previously observed (1924) that in *Pipistrellus pipistrellus*, the uterine glands were active during winter and believed that the secretion of the glands acted as nourishment for the hibernating spermatozoa. Rendez (1929) working with *Vespertilio murinus* and *Placotus auritus* showed that in the males the testes did neither exhibit spermatogenetic activity during spring nor the epididymis contain any active sperms. He also concluded that fertilisation occurred in spring by stored sperms received during autumn copulation.

Harrison Matthews (1937) working on the British horse shoe bats—*Rhinolophus ferrum-equinum* and *Rhinolophus hipposideros minutus*, conclusively proved that copulation occurred in autumn and the spermatozoa stored through winter in the vagina fertilised the ovum which was liberated in spring. He writes, "The occurrence of the vaginal plug in the horse-shoe bats gives some evidence on this question in Rhinolophidae. In specimen taken during the third week of April the vaginal plug was still in position and exactly similar in all respects to that found in the bats up to that date. In addition, there were present, as usual, spermatozoa in the uterine glands and Fallopian tubes. The presence of the plug entirely filling the vagina showed that copulation had been impossible since that plug hardened in the previous autumn. But the particular point of interest was a large and well-developed corpus luteum in the right ovary and the blastocyst was just passing through the uterus. The ovum must, therefore, have been fertilised by one of the spermatozoa stored in the upper part of the genital tract and the spermatozoa must have been deposited in the previous autumn . . .". "These specimens show conclusively that in the Rhinolophid bats the spermatozoa stored in winter can and do fertilise the ovum in spring, some five

months later " This account of Harrison Matthews is conclusive enough But it is a matter of regret that the crucial experiment of keeping inseminated bats segregated until spring, and then examining them for pregnancy was not done by him Further, he did not give any details regarding the condition of the gonads and accessory structures in the males

From the foregoing account it is evident that the view held by these authors is that copulation takes place during autumn and that the spermatozoa are stored in the genital tract of the female till ovulation and fertilisation which occur in spring This observation would apply to *Pipistrellus pipistrellus*, *Nictolus noctula*, *Plecotus auritus*, *Vespertilio murinus*, *Rhinolophus ferrum-equinum*, and *Rhinolophus hipposideros minutus*

The other view that effective copulation, ovulation and fertilisation take place in spring is equally strongly advocated by various workers with respect to the bats of the cold climates Some authors regard this as an exceptional phenomenon occurring either in those bats which have failed to copulate the previous autumn or in the young ones in the first year of sexual life But these researches have so far been confined to the temperate bats only

Vogt (1881) was the first to notice the occurrence of non-pregnant females of *Vespertilio murinus* and *Rhinolophus ferrum-equinum* in spring He believed them to have missed copulation in the previous autumn He does not mention if he observed spring copulation normally occurring in these bats

Rollinat and Trouessart, though they categorically deny spring copulation, still record the instance one male specimen of *Eptesecus serotinus* which, when brought to a warm room in early February, woke up from hibernation and tried to copulate Duval (1895) actually observed spring copulation though not under normal conditions Similar artificial induction of spring copulation was conducted by Zondek (1933) and Caffier (1934)

Hartman and Cuyler (1927) who worked out completely the life-cycle of *Nyctinomus mexicanus* stated that spring copulation was the rule in these American bats They recorded the occurrence of sperms in the uterine tract of the female only in March and in no other season Soon after copulation, fertilisation and gestation followed as in any other mammal However, in a species of *Myotis* (sp) from the same locality sperms were seen in the uterus of the female during winter

Apart from the observations of Hartman and Cuyler quite a large amount of circumstantial evidence has been adduced by the supporters of spring

copulation theory in various species. The conclusions were mainly based on a study of the male reproductive organs. Fries (1879) described that throughout winter and spring the male genital apparatus was full of sperms and the accessory reproductive organs were in full swing of activity. Courrier (1927) made a more detailed study on *Pipistrellus pipistrellus* and recorded that the interstitial cells of the testes and the accessory sexual organs were in full activity during winter though the seminiferous tubules contained no sperms but only spermatogonia and sertoli cells. Rollinat and Trouessart had also observed the storage of spermatozoa in the epididymis and the bladder of the male during winter. Nakano (1928) also recorded the storage of spermatozoa in the epididymis throughout winter. There has, however, not been any conclusive proof that fertilisation occurred by the spermatozoa stored in the genital organs of the males during winter.

Caffier and Kolbow (1931), though they accepted the possibility of fertilisation being effected by the spermatozoa received in autumn copulation, they made the startling discovery that the testes showed spermatogenesis not only in November but also in March in many species such as *Pipistrellus pipistrellus*, *Plecotus auritus*, *Barbastella*, *Eptesecus*, *Myotis*, *Rhinolophus hipposideros*, etc.

The only conclusive evidence in support of effective spring copulation was given by Mary J. Guthrie (1933) who observed the normal occurrence of only spring copulation in many species of North-American insectivorous bats.

There is thus a vast amount of literature available regarding the breeding habits of the insectivorous bats inhabiting temperate and cold climates. Unfortunately there is no complete account of the breeding cycle of the tropical species of microchiroptera and the little knowledge we have is derived only from records of pregnancy. With regard to the copulating season there is practically no information except for the casual observation of the occurrence of visible secondary sexual characters in different seasons in the males of a few species of microchiroptera. Braestrup (1933) recognised in two males of *Chaerophon pumilus* in tropical Africa a large crest of hair on the neck and back and that the scrotum was swollen. This season varied at different places. Thus no generalisation could possibly be made regarding the exact season of copulation and sex cycle on such meagre and casual observations.

A fairly clear account of the breeding seasons of *Miniopterus australis* was given by Baker and Bird (1936). They noticed that, "Conception in this species occurred in the beginning of September". "And the young

were probably born in the second half of December, and the duration of gestation is about 110 days" Further in the middle of August (11th) the examination of the uteri for sperms gave negative results there being no sperms in the uteri or uterine glands There was a large Graafian follicle with much liquor folliculi in the ovary and there were signs of early meta-œstrus condition of the uterine glands The authors recorded that, "Presumably insemination and ovulation would have taken place two or three weeks later"

Their examination of the male specimens substantiated the results of the examination of the female An abundance of spermatozoa in the epididymis was seen in July, August, and September From October onwards there was a decrease in the spermatozoa and till May next the epididymis was practically empty They therefore observed, "One sees clearly that copulation takes place about the end of August and the development of the embryo starts at once Copulation occurs at a time of the year, when the days are beginning to get longer and the temperature is rising, i.e., in spring" "Thus *Miniopterus australis* falls into the same category as *Nyctinomus* at Texas, which Hartmann and Cuyler (1927) showed to copulate only in the northern spring"

After the classical work of Baker and Bird there has been very little work on the tropical bats Harrison Matthews (1941) in his paper 'On the genitalia and reproduction of some South African bats' summarised that in the tropical species there was nothing comparable to the winter hibernation of the temperate bats Only in the case of *Miniopterus dasythrix* he mentions "Possibly that impregnation had taken place weeks, or even months, previously and that spermatozoa had been stored in both sexes as in some European bats" However, in the "summary", he observed, "for early in July the females were pregnant with only blastocysts, indicating mating at a season corresponding with the earliest beginnings of the southern spring It is of course possible that insemination took place in the preceding autumn and that fertilisation or development had been delayed, as in some bats of the temperate regions, but it does not appear to be likely because there is no evidence that this species hibernates"

We thus see that though a great controversy exists regarding the exact seasons of copulation and fertilisation in the insectivorous bats of the temperate and cold climates, there seems to be entire agreement among the workers on the tropical bats, that copulation occurs in early spring and is immediately followed by fertilisation and gestation

My study of the reproduction of the South Indian Vespertilionid bat *Scotophilus wroughtoni* (Thomas) confirms the above view. There is no storage and hibernation of spermatozoa.

#### MATERIAL AND METHODS

Bats of this species were collected round about Bangalore from the forests of Hoskote (about 17 miles east of Bangalore). Some were also collected at Seringapatam about 75 miles west of Bangalore. This does not alter the results of the work as both the localities conform to the same plan of breeding. *Scotophilus wroughtoni* is essentially an arboreal species living inside hollows of large trees. A few excursions were made to the caves and dungeons near Bangalore and Seringapatam, but at no time could we collect a single specimen of this species though many other species were collected in large numbers. Further, it appears that this particular bat is always found to live in association with two other species of insectivorous bats—*Scotophilus temminki* and *Taphozos longimanus*, for all our collection of bats included all the three species.

*Scotophilus wroughtoni* is a fairly large bat with a brown coloured belly and the back of darker hue. The tail projects slightly beyond the interfemoral membrane. The bats hang down from projections inside the hollows of trees. They were caught by using a net. They are ferocious and are to be handled with care, as they bite otherwise.

The specimens were killed by chloroform and immediately dissected and the genital structures removed. The carcasses are all preserved in formalin for further study. The reproductive organs were fixed in various fluids. But Bouin's picro-formal gave the best results. After fixation the material was transferred to 70% alcohol. In the females the mammary glands and in the males the adrenal bodies were similarly fixed. Serial sections of the ovaries, Fallopian tubes, the uterus and vagina were taken in all cases where there was no visible signs of pregnancy.

An account of the changes in the male reproductive and detailed histological oestrous changes in the female will be dealt with in the next part.

Collections of bats began in the month of May 1945 and is still being continued to the present day. Attempts were made to collect as many times as possible in all months of the year to complete the data regarding the breeding habits.

Table I gives the record of the collection of the bats so far made.

TABLE I

*Scotophilus wroughtoni* (Thomas) Monthly record of collections

Month	Males	Females	Pregnant Female
January	3	5	No pregnant
February	5	1	
March	2	4	Early morulae
April	2	40	Early blastocysts
May	4	30	Advanced pregnancy
June	2	2	Full term
July			
August			
September	1	1	Non pregnant
October	1	1	
November	1	2	
December	2	17	

The above numbers do not probably indicate the sex ratio because in practically all our collections the females outnumbered the males. One thing worth recording is that during the months of January and February there were a greater number of males than during the other months. This fact taken with the other things might probably indicate that during this period only do the males and the females live together while at other periods males live segregated from the females. No definite generalisation is, however, possible at this stage.

## OBSERVATIONS

(a) *Number of embryos in a litter*—At each pregnancy there are two embryos, and each ovary shows a corpus luteum—a fact which is of very rare occurrence among the microchiroptera single embryos being the rule. Two embryos were also observed in *Scotophilus temminckii*. Occurrence of double embryos was noticed by Ramaswamy (1933) in another Vespertilionid bat, *Vesperugo leisleri* (Kuhl). Harrison Matthews (1942) states, "One of the most interesting characters of the female genitalia in the microchiroptera is the bilateral asymmetry which occurs in varying degrees of intensity." "Most bats, except those of the family Phyllostomatidae, have a bicornuate uterus, but nearly always bring forth only one young at a birth, consequently as a rule, only one uterine cornu is occupied by pregnancy. It has been found in very many species of different families that there is a constant tendency for the right side of the genitalia to be the functional one. In many European Vespertilionids, although pregnancy can occur on either side, the majority of pregnancy has been found in the right cornu." In *Rhinolophus hipposideros* he has shown that "the left ovary appears to be degenerate and never to produce mature ova, the pregnancy being always

on the right side" (Matthews, 1937 a) I have also observed that in many of the species of microchiroptera that I collected there was always a single embryo in the uterus. Thus, *Scotophilus wroughtoni* differs from a majority of the microchiroptera in having two embryos in the litter. However, there is one specimen in my collection in which the left ovary shows two masses of corpora lutea and two unimplanted blastocysts in the left horn of the uterus but at different levels. Probably double implantation never occurs in this species in the same uterine cornu, the ovum, before or after fertilisation moving into the other cornu for implantation even in those exceptional cases where double corpora lutea occurred in the same ovary. This surmise seems to be correct, because after the establishment of the placenta, there was no case where a double embryo occurred in the same uterine cornu. A similar migration of the ovum from the ovary to the opposite uterine horn for implantation has been described as a normal occurrence in the case of *Miniopterus dasythrix* (Temm.) (Harrison Matthews, 1942)

(b) *The breeding seasons*—Pregnancy records show that the female has a very sharply defined annual breeding season. Pregnancy was observed only from the 22nd March upto about the end of June, and at no other period was a pregnant specimen collected. This seems to confirm the observations of Baker and Bird (1937) on *Miniopterus australis* which "presents a very sharply defined annual breeding season", where pregnancies occurred only during the months of September, October, November and December, and no pregnancy during the other months of the year. This is also the case in all the species of temperate and cold climates so far examined by various authors. Marshall (1922) states "it does not appear to be known whether the poly-œstrous condition ever occurs in bats". However, Ramaswamy (1933) observing pregnant uteri in early January in *Vesperugo leisleri* (Kuhl) suggests, "It is also possible that there is another season when these begin to breed". "It looks as though after a very short anæstrum following the summer gestation the pro-œstrous cycle again commences ending in the copulation of the females in cold weather". Harrison Matthews is the only other author to record a poly-œstrous condition in *Nycterus leuola* (Thos.) and *Nycterus hispida* (Schreb.), wherein he observed pregnancies in lactating bats and concluded, "The quick succession of pregnancies also points to the possibility that this species, unlike all other bats as far as they are known, may be poly-œstrous". But *Scotophilus wroughtoni* without any doubt has only one annual breeding season.

(c) *The œstrus and copulation*—The uterus which shows inactive glands as late as November suddenly springs to activity in February, and the glands

hypertrophy with a definite increase in the vascularisation of the uterine submucosa (Fig 1) A very careful microscopic examination was made to detect the presence of spermatozoa but in specimens collected in November, December, January and February no sperms were seen in the uterus, vagina, or the Fallopian tubes The ovaries of the February specimens showed great activity and exhibited a large number of developing Graafian follicles (Fig 2) Ovulation does not certainly occur upto the 10th of February Examination of specimens collected on the 24th of March clearly shows that ovulation not only has occurred but in all females early morulae were present These were lying loose in the uterine lumen Further the vagina showed large numbers of degenerating spermatozoa Another curious fact noticed was that out of the twelve females collected on 1st of April all were pregnant—pregnancy being recognised only after careful microscopic examination of the uterus, and in all the cases the blastocysts were in the same stage of development, lying loose in the uterine cavity (Fig 3) This fact clearly shows that fertilisation occurred in all the specimens at about the same period, if not on the same day This, taken along with the fact that the females collected on the 10th of February showed no spermatozoa, indicates that the period of copulation is also very sharply marked, and it must have occurred between the 10th of February and the 24th of March Judging by the age of the morula on the 24th of March one can easily place the time of fertilisation somewhere about the third week of March It is, however, not possible to clearly decide whether copulation occurs before or after ovulation because I have unfortunately no collection made during the 1st, 2nd, or the 3rd week of March But probably copulation might have occurred a day or two after ovulation because no sperms were seen in the Fallopian tubes while sperms were quite abundant in the uterine lumen in the specimens collected on 24th March There is no instance of a tubal ovum in my collection

Pregnancy was noticed in all specimens collected between the months of April and June at progressively advanced stages The June embryos were far advanced in development, though not of full term Parturition can safely be placed at the last week of June or the first week of July The period of gestation thus extends from 105 to 120 days

In the whole of my collection there was no instance of pregnancy in a lactating female

(d) *Age and growth*—All females captured during April, May and June were pregnant without a single exception This seems to be a very interesting feature, and Baker and Bird omit to make a mention of this.

Rollinat and Trouessart working on some of the Rhinolophid bats observe "frequently the young females in their second year do not experience œstrus, and consequently their first œstrus does not occur until their third autumn" These authors divided their material into four groups virgin animals in their first autumn, virgins in their second autumn, animals experiencing their first œstrus, some in their second and some in their third autumn and parous animals, some in their third and some at least in their fourth autumn" (Harrison Matthews, 1937)

Harrison Matthews (1937) also records "Young bats do not reach their first œstrus until their second autumn when they are at least 15 months old Parous bats will at least reach their second œstrus when they are 12 months older, and at least 27 months of age By the time when they have weaned their second young one they will be 34 months old" He thus endorses the view-point of Rollinat and Trouessart

He also tried to determine the age of his specimens by their pregnancy records He says "the present series of specimens show clearly that both species of British horse-shoe bats normally do rear a second young one, and consequently must reach an age of at least three years" "Further on purely theoretical grounds these bats must live to an age of at least four years, because each pair must produce more than two young in its life-time, to allow for wastage, if the species is not to become extinct Females must therefore produce at least three young each, and accordingly reach an age of four years"

Theorising on similar lines regarding *Scotophilus wroughtoni*, as there is no non-pregnant female during the gestation season, i.e., April to June, and also as there is no record to show that the bats might become pregnant during any other month of the year, as it is monœstrous, the species must get into sexual activity in its first year and become pregnant Thus, before it has completed its one year of age, it will have given birth to young ones Furthermore, the growth of the young one is very rapid as is indicated by the ovaries of the specimens collected in February That the non occurrence of non-pregnant females during the months of April, May and June, during 1945 and 1946, cannot be an accident It must be presumed that all females had become pregnant All these facts clearly indicate that the young born in the month of July the previous year get into sexual activity during next February and March

Purely on theoretical grounds, and fitting with the conclusions of Harrison Matthews, these bats must produce at least three young ones each, to perpetuate the race, and hence must at least live for two years As this

particular species under study bears two young ones in each litter it is quite possible the bat becomes pregnant at least twice in its life-time. Further, there is no indication to show that the bat had become pregnant more than twice as revealed by the residual placental discs. Thus taken along with the fact that the bat experiences an annual breeding season indicates that it must live for atleast two years and that it may not become pregnant a third time. Furthermore, Anderson (1917) states that the length of the period of immaturity will, as a general rule, in some vague sort of way, enable us to form an opinion of the normal age the individual is destined to obtain, a mammal which quickly becomes full grown will probably have a rather short series of years to live as adult, and *vice versa*." Anderson places the age of the bat *Rhinolophus rouxi* "at five or six years as the extreme possible age of the bats", as calculated from their tooth-wear. Taking all these things into consideration *Scotophilus wroughtoni* does not probably live as long as *Rhinolophus rouxi*, or the British horse-shoe bat, which are supposed to have a longevity of about four and a half to five years but might live, without doubt, up to about three years and probably not more. A more clear and definite figure will be arrived at by examining the tooth-wear on the same lines as Anderson did, which will be shortly undertaken.

#### CONCLUSIONS

Two very important facts are recognised by the study of the reproductive phenomena in *Scotophilus wroughtoni*. In the first place, there is a very sharply defined breeding season confined to about the middle of March, and secondly all the females collected during the months of April, May and June are pregnant. In an unvarying tropical climate the occurrence of an annual monœstrous condition is by itself remarkable, and much more so in the case of a bat which is confined to the hollows of trees—considering the non-conductivity of the wood to temperature, and thereby living under an almost constant environmental condition throughout the year. Baker and Bird (1936) also make a similar discovery in the bats of New-Hebrides and come to an identical conclusion. The seasonal change in the temperature alone does not probably determine the onset of the breeding activity, as was supposed by a few of the early workers on the bats of temperate and cold climates. Furthermore, too much stress cannot be laid on the factor of light and of the lengthening of the day, considering the fact that the bats are essentially nocturnal creatures. Unless experimental data is available regarding the influence of light on the breeding habits of bats, this conclusion cannot be accepted at present.

As copulation is immediately followed by fertilisation and gestation the problem of winter hibernation of the spermatozoa, as occurs in the bats of cold climates, becomes unnecessary

#### SUMMARY

1 A study of the literature in the reproduction of bats reveals that there are two types of sexual phenomena exhibited by bats, some bats experiencing a definite hibernation during winter after copulation in autumn, and others where copulation is immediately followed by fertilisation and gestation in spring. The bat *Scotophilus wroughtoni* does not show any evidence of a winter "sleep" and thereby falls into the second category.

2 *Scotophilus wroughtoni* has a sharply defined breeding season, copulation occurring at about the middle of March and followed immediately by fertilisation and gestation.

3 The period of gestation is about 105 to 115 days.

4 The age which this bat attains, as determined by its pregnancy records, may safely be placed at about three years.

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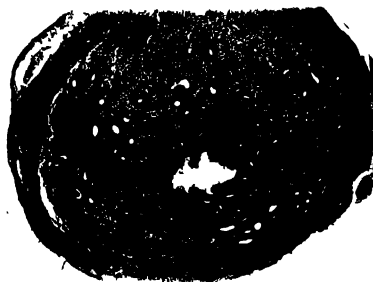


FIG. 1 Transverse section of the uterus of a specimen collected on 10th February showing great hypertrophy of the glands at the onset of the breeding season

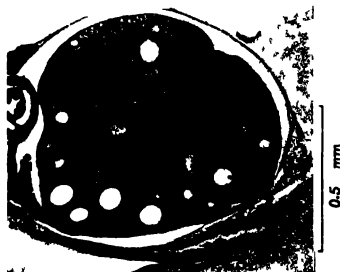


FIG. 2 Transverse section of the ovary from a specimen collected on 10th February showing the great activity of the germinal epithelium. The ovary presents a large number of graafian follicles. Ovulation has not yet occurred



FIG. 3. Transverse section through the ovary of a specimen collected on 1st April showing a free unimplanted blastocyst lying loose in the uterine lumen. All specimens collected on this date show blastocysts in the same stage of development.

## UNDESCRIBED MALES OF TWO SPECIES OF GALL MIDGES\*

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THE present paper deals with the descriptions of males of two species of Indian gall midges (Itonididae: Diptera). Both the species were first described from female specimens collected by the author from Travancore, the following descriptions are based on males collected on subsequent dates.

The *Allotype* males are to be deposited in the Zoological Survey of India, Benares Cantt.

### *Trichopteromyia Manu Nayar*

The author first described the species (Nayar, 1944) from female midges collected at light from Trivandrum. The following is a description of the male collected from Trivandrum on a later date at light.

*Male*—0.6 mm long, brownish-red. Eyes confluent above. Palpi quadriarticulate, as in female, scaled, basal segment cylindrical, shortest, terminal segment longest, ovate, more or less pointed distally. Antennæ with fifteen segments, bearing whorls of long setæ, structure more or less similar to that of the female, first segment broadest, hemispherical, the rounded portion attached to the head, second segment globose, slightly compressed, its diameter three-fourths that of the broad end of the first, the attachment to the basal segment at an extra-central point, third a little less in diameter than the second, the stems increasing in size distally, thirteenth segment with a stem as long as the thickness of the third, the enlargement one-third as long as the length of the segment, fourteenth segment with a button-like process representing the fifteenth segment, the enlargement more than two and a half times as thick as the fifteenth. Mesonotum smoky brown. Wings hyaline, as in the female. Halteres blackish brown. Legs smoky-brown, moderately hairy, hindlegs longest. Claws simple, as long as the empodium. Abdomen reddish and dark scaled. Genitalia small, basal clasp segment cylindrical, oblong, three-fifths as broad as long, terminal clasp segment small, sting-like, oblong-ovoid,

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\* Part of thesis approved for the Ph. D. degree of Travancore University.

slightly longer than the breadth of the basal clasp segment with a pin like pointed tip slightly hairy style small rounded at tip about as long as the basal clasp segment

Type locality Trivandrum Collected at light on various dates in June 1944

This species differs from the genotype *Trichopteromyia modesta* Williston in the smaller size of the body the quadriarticulate palpi and the antennal characters

*Prolasioptera aschynanthus perottetti* Mani

Mani (1943) described the species from female specimens bred by me from stem galls on *Aeschynanthus perottetti* A Dc from Pampadampara Hills in the High Ranges of Travancore Subsequently I was able to rear males also The following is a description of the male

Male 2 mm long brownish Palpi as in the female Antennæ incomplete similar to that of the female third and fourth segments fused together Mesonotum dark brown Wings and halteres as in the female Abdomen comparatively stout Genitalia (Fig 1) small basal clasp seg

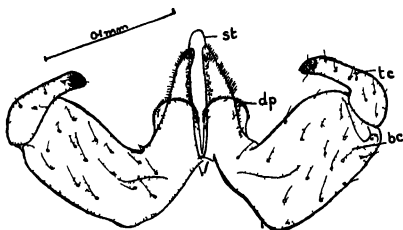


FIG 1 Male genitalia of *Prolasioptera aschynanthus perottetti* Mani  
bc basal clasp segment dp dorsal plate st style tc terminal clasp segment

ment oblong its length slightly less than double its own breadth terminal clasp segment roughly rounded at its joint with the basal clasp segment, malleiform with fairly serrated and moderately chitinated tip length about two-thirds that of the basal clasp segment broadest part about one third its own length, style as long as the basal clasp segment, dorsal plate deeply cleft and truncated distally

Type locality Pampadamparai Hills in the High Ranges of Travancore  
Reared from stem galls on *Aeschynanthus perottetti* A. Dc., in August 1944

This species is easily distinguished from the other Indian species *Prolasioptera annandalei* Mani, by the longer body and the twenty-one segmented antennae

Nayar (1945) described the stem gall on *Aeschynanthus perottetti* A. Dc., from which the midges were bred. A leaf gall has also been collected by him on the same plant, from Pampadamparai Hills and is believed to be produced by the same midge. I give below the description of the gall.

*Leaf gall*—16 mm long, 5 to 9 mm across irregular, green with a violetishbrown tinge, soft, succulent, somewhat granulated inside, hypophyllous, with a number of larval chambers inside.

Locality Pampadamparai Hills

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# CYTOGENETICAL STUDIES IN SESAMUM

Part I Cytology of the Parents, *Sesamum orientale* Linn and *Sesamum prostratum* Retz and the Cytology of the Sterile Hybrid between them and of the Fertile Amphidiploid

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CONTENTS	PAGE
I INTRODUCTION	236
II MATERIALS AND METHOD	238
III CYTOLOGICAL OBSERVATIONS—	
(a) <i>Sesamum orientale</i> Linn	238
(b) <i>Sesamum prostratum</i> Retz	244
(c) Sterile hybrid	245
(d) The amphidiploid	249
IV DISCUSSION—	
(a) Nucleolus—its behaviour and persistence	252
(b) Interspecific hybridisation—a guide to ancestral homology	253
(c) Artificial synthesis of a new species	257
(d) The possible origin of the cultivated Til <i>Sesamum Orientale</i> Linn	265
(e) The possible origin of <i>Sesamum prostratum</i> Retz	268
V SUMMARY	271
VI LITERATURE CITED	272

## I INTRODUCTION

PEDALINEAE is a very small family of annual and perennial herbs, distributed mainly in the eastern tropics Bentham and Hooker (1885) record only two genera in India, *Petalum* and *Sesamum Martynia* which is also included in this family is reported to be an American weed introduced into India

The genus *Petalum* is represented only by a single species, *Petalum murex* Linn which grows wild in waste lands of South India The genus *Sesamum* is represented by both perennial and annual species Bentham and Hooker (1885) have recorded only three Indian species of *Sesamum*,

*Sesamum orientale* Linn and *Sesamum prostratum* Retz, and *Sesamum laciniatum* Klein Among these *Sesamum orientale* is an annual whereas the other two species are perennials *Sesamum prostratum* grows wild on the sand dunes near about the shores of Madras while *Sesamum laciniatum* thrives on the barren rocks of the Deccan hills

Little cytological and cytogenetical data have been recorded for the two genera, *Pedalium* and *Sesamum* The genera *Pedalium* and *Martynia* have been investigated cytologically and cytomorphologically in this laboratory as part of the extensive cytogenetical investigations in the family Pedaliaceæ (Srinivasan, A R, 1942) The chromosome number of *Pedalium murex* Linn was determined to be  $2n = 16$  and its life-history was worked out with special reference to the development of the endosperm haustoria in the female gametophyte The species *Martynia diandra* Glox, which grows wild in these parts, was also investigated and its diploid chromosome number was determined to be 32

According to Schnarf (1931) the genus *Sesamum* has been investigated with reference to the occurrence of endosperm haustoria, by Balicka Iwanowska (1899)

Morinaga *et al* (1929) determined the somatic number of *Sesamum orientale* ( $2n = 26$ ) Nohara (1934), Richharia and Suguira (1936) have reported the meiotic number of the same species (*Sesamum orientale*) to be 13 The present cytological investigation goes to confirm the numbers previously recorded

The perennial species *Sesamum prostratum* has never been investigated either cytologically or cytomorphologically till recently when its meiotic number was determined to be 16 by Ramanujam (1941) This number has been confirmed in the present investigation

While cytogenetical investigation in this laboratory had proceeded more than half way through, a short note appeared in *Current Science* recording some data in respect of hybridisation between the cultivated and wild species of *Sesamum* (Ramanujam, 1942)

In the course of the present investigation the chromosome number of *Sesamum laciniatum* Klein, another wild species, was determined for the first time in this laboratory to be  $2n = 28$  (Raghavan and Krishnamurthy, 1945)

Interspecific hybridisation between *Sesamum orientale* and *Sesamum prostratum* has been in progress for some years now in this laboratory and the sterile hybrid derived therefrom was made fertile artificially by the induction of amphidiploidy, through the application of Colchicine The cytology

of the sterile hybrid, its meiotic irregularity and the ultimate formation of abnormal sporads are detailed in this paper. The regular meiosis of the fertile hybrid after the artificial induction of amphidiploidy has also been included.

## II MATERIALS AND METHOD

Crops of *Sesamum orientale* belonging to the local red-seeded strain were raised from time to time in the University Botanical Gardens, Annamalai-nagar. Seeds of *Sesamum prostratum* were collected from various localities, especially from Adyar beach, Madras and Coimbatore. Seeds were sown in small pots and were kept in a warm room where they germinated early. Root tips from *Sesamum orientale* were available within 60 hours after sowing whereas those of *Sesamum prostratum* could be obtained only after 5 or 6 days.

Good root tips of the parents and the hybrid could easily be obtained without injuring them since they were sown only on the upper layer, just below the soil. Various fixing fluids were used and fixing was done at various intervals of time. Maximum mitotic activity was observed at mid-day. The fixatives used were Karpechenko's modification of Nawaschin's chrome-acetic-formalin, Irene Manton's modification and Muntzing's formula. Of these fixatives, Irene Manton's modification proved to yield good results. Prefixation in Carnoy's fluid was done in all cases to aid proper fixation.

Flower buds were fixed at various hours of the day. Here also mid-day fixing showed good results. Irene Manton's formula was used. Materials were imbedded in paraffin of melting point 52° C using chloroform as the paraffin solvent.

Sections were cut at thickness varying from 12 to 15 microns and stained in Newton's Iodine Gentian Violet and Haidenhain's Iron Alum Haematoxylin (Chamberlain, 1932). Right stages of anthers were determined before fixing by aceto-carmin examination. Drawings were made at table level using Abbe drawing apparatus and their respective magnifications are indicated.

## III CYTOLOGICAL OBSERVATIONS

### (a) *Sesamum orientale* Linn

*Somatic chromosomes*—Fig 1 shows a somatic metaphase plate of *Sesamum orientale* with 26 chromosomes thus confirming the previous record made by Morinaga *et al* (1929). There is no disparity in the size of the chromosomes in the somatic complement. Almost all the chromosomes would appear to be characterised by terminal centromeres. An analysis

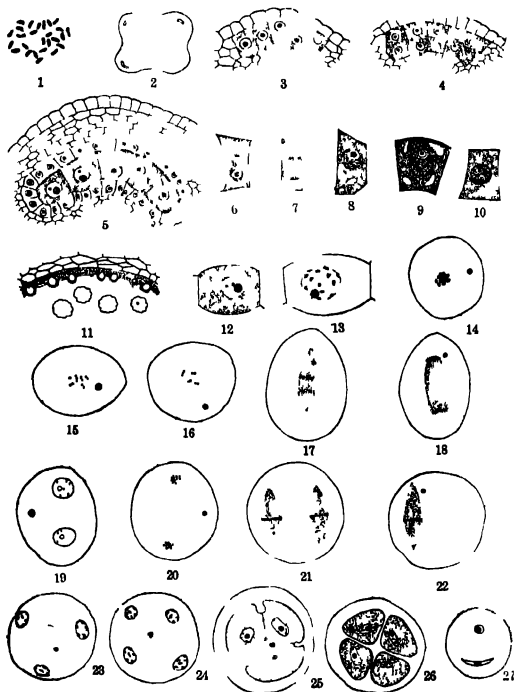
of the chromosome complement on the basis of their morphology was not therefore considered useful and hence not attempted

**Microsporangial development**—The archesporium of the anther consists of groups of 5 or 6 hypodermal cells in the four corners of the anther as seen in Fig 2 Multicellular archesporia are by no means uncommon In the closely related genus (*Petalum*, such extensive archesporia have been recorded (Srinivasan, A R, 1942) They have also been found in the genus *Nicotiana* (Raghavan and Srinivasan, A R, 1941 a)

To begin with, the single row of archesporial cells (Fig 3) divide periclinally resulting in the formation of two layers of cells, outer forming the primary parietal cells and the inner primary sporogenous cells (Fig 4) In the mature anther, the parietal tissue consists of four or five layers, the innermost of which functions as the tapetum (Fig 5)

It is interesting to note, in this connection, the behaviour of the tapetal nucleus To start with, the tapetal cells are uninucleate and contain dense cytoplasm (Fig 6) At a later stage, the nucleus of the tapetal cell shows signs of division resulting in the formation of two nuclei (Fig 7) Division of the tapetal nucleus takes place even before the nucleus of the pollen-mother cells enters into the early stages of meiosis After the division, two nuclei are formed within the same tapetal cell, which, after some time, come together Fig 8 shows two nuclei after division In most cases, it has been observed that these two nuclei of the tapetal cells do not remain separate, but that they tend towards fusion Fig 9 shows a big binucleate tapetal cell showing the fusion of the two nuclei There are stages when more than two nuclei are formed by repeated division and ultimately fuse with one another resulting in a tapetal cell with the nucleoli of all the nuclei so fused. Thus in Fig 10 we find that there are 5 nucleoli within the single nucleus, indicating thereby that the nucleus of the tapetal cell has divided giving rise to 5 daughter nuclei all of which have fused together to form the five-nucleolated nucleus Cooper (1933) recognises three types of tapetal cells (1) in which the tapetal cells remain uninucleate, (2) in which they are binucleate and (3) in which they become plurinucleate The present case in *Sesamum orientale* belongs to the third type described by Cooper Such plurinucleate tapetal cells have been a common feature in many of the angiospermic genera investigated In *Gynadropsis* (Raghavan, 1938), in *Chenopodium* (Bhargava, 1936), in *Portulaca* (Raghavan and Srinivasan, A R., 1941b), in *Astercantha* (Rangaswamy, K., 1941) and in *Crescentia* (Venkatasubban, 1944) they have been observed to occur

The division of the tapetal cells has been found to be mitotic in this species (Fig 7) It was at one time believed that the division of the tapetals



Figs 1-27

TEXT FIGS 1-27 *Meiosis in Sesamum orientale* Linn.—Fig 1 Somatic complement of *Sesamum orientale* showing 26 chromosomes Fig 2 Section of a very young anther showing the hypodermal band of archesporium  $\times 150$  Fig 3 A band of primary archesporial cells  $\times 150$  Fig 4 Division of the archesporial cells  $\times$  primary wall cells and primary sporogenous layer  $\times 150$  Fig 5 Mature anther showing five wall layers the innermost forming the tapetum  $\times 150$  Figs 6-10 Tapetal cells showing stages of nuclear division and fusion  $\times 400$  Fig 6 Binucleate tapetal cell Fig 7 Mitotic division of the nucleus Fig 8 Uninucleate tapetum Fig 9 Fusion of the two nuclei in a single cell Fig 10 Cell showing 5 nucleoli within a single nucleus Fig 11 Degeneration of the tapetal layer at the pollen grain stage Fig 12 Resting nucleus of pollen mother cells showing the budding off of spherical bodies  $\times 1000$  Fig 13 Diakinesis showing 11 bivalents  $\times 1000$  Fig 14 Prometaphase The persistent nucleolus is towards the one side of the cell  $\times 3000$  Figs 15 and 16 Metaphase groups showing 13 bivalents in secondary association 1, 4 and 2, The persistent nucleolus is away from the equatorial region  $\times 3000$  Figs 17 and 18 Anaphase separation with the persistent nucleolus going ahead of the chromosomes Fig 19 Interphase nuclei with the persistent nucleolus towards one side Fig 20 Metaphase II showing 13 chromosomes and the separately lying persistent nucleolus Fig 21 Spindles of Anaphase II stage lying parallel Fig 22 Spindles of Anaphase II stage lying at right angles Fig 23 Pollen mother cell showing 3 of telophase nuclei lying in one focus and the fourth in another Fig 24 Pollen mother cell showing 4 telophase nuclei all lying in one plane The persistent nucleolus is in the middle Fig 25 Tetrahedral type of tetrad on the process of furrowing The persistent nucleolus is included in one of the tetra cells Fig 26 Isobilateral tetrad cells arranged in one plane Fig 27 Two-celled pollen grain at the time of shedding All figures have been drawn at a magnification of  $\times 3000$  unless otherwise stated

nucleus is amitotic Rocen (1927), in *Portulaca*, and O'Neill (1920), in *Datura* But that it is through ordinary mitosis has been observed critically and confirmed by Raghavan (1938) in connection with his investigations on *Gynandropsis* In several other genera investigated in this laboratory, mitosis was found to be the rule It would thus appear safe to generalise that tapetal nuclear division is through mitosis

**Meiosis**—The microsporangial tissue consists of a single row of five or six microspore mother cells (Fig 5)

In the resting condition of the nucleus of some of the pollen mother cells, in addition to the big darkly stained nucleolus, small bodies similarly stained but smaller than the nucleolus have been found to occur (Fig 12) In one and the same locus of the anther, some pollen mother cells show these bodies while in others they are conspicuous by their absence Similar bodies have been recorded in the pollen mother cells of various genera, viz., in *Oryza* (Nandi, 1937), in *Hibiscus mutabilis* (Majumdar and Datta, *et al.*, 1934), in *Cicer arietinum* (Iyengar, N K., 1939) and in *Oenothera rubrinervis* (Gates, 1908) Most of them regard these spherical bodies as extrusions from the nucleoli and consider them to be intermediate stages during the transference of chromatin material from the nucleoli to the chromosomes.

These bodies persist throughout the stages of meiosis right up to the tetrad stage and they have been observed to be included in one of the tetrad cells (Fig. 26)

The possibility of these bodies being chromosomes or their fragments is ruled out for the following reasons: namely (1) they do not take up any particular position with respect to the cell and are apparently not attracted by forces of attraction or repulsion which are presumed to be responsible for the chromosome movements observed during nuclear division, (2) they are perfectly spherical in shape and homogeneous in structure, (3) they do not undergo any change in their shape or size during meiosis, (4) they are larger than the bivalents or chromosomes at any stage during meiosis, though they are smaller than the prophase nucleolus. That these bodies are nucleolar in origin has been confirmed by positive evidence also. The spherical bodies get stained to the same extent as the nucleolus itself. These bodies seem to bud off from the big prophase nucleolus (Fig. 12) and at various stages, the connection of these bodies with the big nucleolus has been observed clearly. Kumar and Abraham (1942) on their observation in *Sesamum*, call these bodies secondary nucleoli, a name suggestive of their origin.

The behaviour of these spherical bodies during meiosis is indicated at the different stages thereof. In some cases, these bodies were not to be found in the tetrad cells. It is believed that they disappear in the cytoplasm of the pollen mother cells when the tetrads are forming. Probably in most cases they disappear from the scene failing which they are included in one of the tetrad cells.

At diakinesis, the 26 chromosomes of the somatic complement are seen to form 13 bivalents (Fig. 13). These 13 bivalents are mostly of the rod type. They are distributed on the periphery of the nucleus. All the pairs are dispersed at equal distance from each other. This equidistant spacing of the bivalents, according to Lawrence (1931), is due to a repulsion phase which begins at early diakinesis and continues till mid-diakinesis.

The converging movement of the bivalents begins at mid-diakinesis and continues until the bivalents are in close association in the centre of the nucleus. The main nucleolus disappears though the nucleolar bit persists in the form of a spherical body, a little away from the clumped mass of bivalents (Fig. 14).

First metaphase follows prometaphase. The 13 bivalents are arranged on the equatorial plate and are evenly distributed unlike in the case of the sterile hybrid where they are scattered. The bivalents exhibit secondary association, frequently resulting in a number of groups. The maximum

association observed is 1, 4, and 2, thus bringing the total number of groups to 7. Probably this would suggest that the original basic number of the genus is 7. Based on this suggested basic number, the possible origin of the cultivated species has been discussed at the end of the paper. Figs 15 and 16 show the metaphase plate exhibiting the phenomenon of secondary association. It may be noticed that the persistent nucleolus now takes up a position away from the dividing bivalents. This would naturally indicate that it does not get itself involved in the division of the bivalents and thus rules out the possibility of its being chromosomal in nature.

After the metaphase stage, the chromosomes are subjected to anaphasic separation. Anaphase, in this case, is quite normal and the chromosomes disjoin with marked uniformity. The persistent nucleolar body, which is lying away from the equatorial plate during metaphase, is now to be seen at one of the poles. Presumably it has already gone ahead of the chromosomes towards the poles. Figs 17 and 18 represent the normal anaphase separation and the persistent nucleolus lying at one of the poles.

At each pole, after anaphase, the chromosomes arrange themselves in groups and organize themselves into the interphase nuclei (Fig 19). Now the nucleolus makes its appearance at both of the interphase nuclei. The chromosomes are more or less uniformly spaced. Such uniform spacing of the chromosomes in the first telophase nucleus has been recorded in *Angelonia* (Raghavan and Srinivasan, V. K., 1940), in *Oenothera* (Gates, 1909) and in *Gynandropsis* (Raghavan, 1938). Gates attributed the uniform spacing of the chromosomes at interkinesis to a mutual repulsion, and the clumping at early telophase, due to attraction. But the "medium in which bodies float frequently change their qualities of attraction and repulsion and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float". No partition wall is formed between the daughter nuclei nor is the resting stage reached by the interkinesis nuclei (Fig 19). The persistent nucleolus in some of the cells at this stage occupies a place towards the side of the pollen mother cell while in some cases they were found near one of the telophase groups (Fig 19).

When second metaphase sets in, the interphase nuclei at either pole lose their nuclear membrane and their nucleoli. The persistent nucleolar body is not involved in it (Fig 20). At this stage the 13 haploid chromosomes are seen arranged uniformly at the poles.

The second metaphase chromosomes undergo normal disjunction and they reach the poles without exhibiting any irregular phenomenon like bridge formation or fragmentation. Figs 21 and 22 show the second ana-

phase stage and the persistent nucleolus may be seen on the spindle fibres of one of the anaphase sets. Here also there is an indication by its mere position at the poles that it precedes the chromosomes. The possible causes for the migration of the nucleolus to the poles ahead of the chromosomes have been discussed further below.

The organisation of the spindle during anaphase separation may take place in two ways. In some cells, the spindles lie parallel to each other as in Fig 21. In others, they lie at right angles to each other so that in one focus, one anaphase group will show the side view of the chromosomes while the other will show the polar view as in Fig 22. The nature of the tetrads will obviously depend upon the position of the spindles during anaphase. If the spindles are parallel then the four telophase nuclei lie in the same plane (Fig 24) leading to the formation of iso-bilateral tetrads (Fig 26). If they are at right angles as in Fig 22 and 23 then the arrangement is tetrahedral (Fig 25). Both the types of tetrad arrangement have been noticed in *Sesamum orientale*. The persistent body at this stage is found to be included in one of the tetrad cells (Figs 25 and 26). Simultaneous furrowing takes place during the formation of the tetrad from the periphery towards the centre. Fig 25 shows a stage in the process of furrowing. Due to the simultaneous furrowing all the four tetrads are organised simultaneously.

The pollen grain at the shedding stage shows a crescent-shaped generative cell and a small tube cell (Fig 27). The pollen grains are uniform in size and their wall shows ridges and furrows. All the grains are viable and germinate rapidly in sugar agar cultures. Plate I, Fig 1, shows a microphotograph of the pollen grains of *Sesamum orientale*.

(b) *Sesamum prostratum* Retz

The somatic complement is made up of 32 chromosomes (Fig 28). They are uniform showing no disparity in size or morphology. All the chromosomes of the somatic complement show terminal constriction.

The microsporangial development and the general outline of meiosis conform to the details already described for *Sesamum orientale*. The tapetal nucleus and its behaviour is also similar to that of *Sesamum orientale*. Fig 29 shows three tapetal cells in the process of division. In the first cell, anaphase has just set in. In the second cell, the chromosomes have separated and are reaching the poles. In the third cell, two nuclei have ready formed. These figures confirm the mitotic nature of the division of the tapetal cell.

Almost all the pollen mother cells, in their resting condition, show the peculiar phenomenon of nucleolar budding (Figs 30 a to f). The nucleolar

buds so formed vary in number and it is found that within a pollen mother cell, in some cases as many as 7 buds were seen (Fig 30 f). But these buds have not been found to persist as in the previous species through meiosis.

Further meiotic stages are normal. Fig 31 shows first metaphase plate showing 16 bivalents.

### (c) Sterile Hybrid

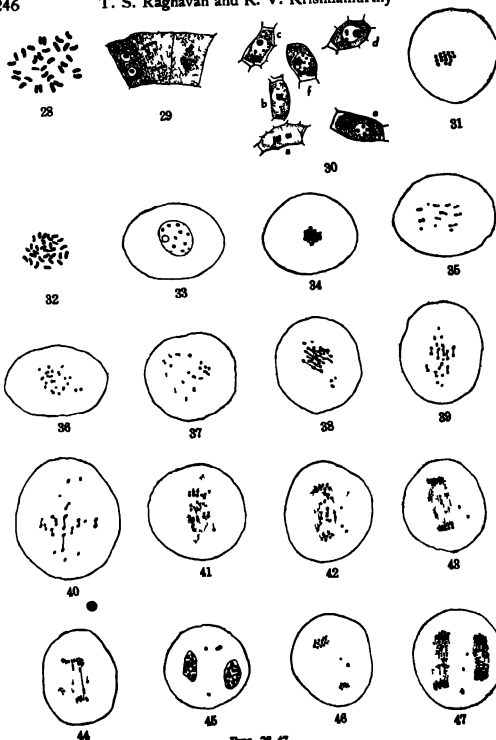
The diploid complement of the  $F_1$  hybrid shows 29 chromosomes (Fig 32). Somatic cells of the root tips and of flower buds were examined for purposes of confirmation. Of the 29 chromosomes 16 are derived from the *prostratum* parent and 13 from the *orientale* parent. Since the parental complements showed no morphological disparity among themselves no morphological distinction between these two sets of chromosomes could be recognised in the hybrid complement.

**Meiosis.** The origin and development of the microsporangium, tapetal behaviour, etc., present no deviation worth any special mention. There is also the same nucleolar budding which was a characteristic feature of both the parents. As many as seven bodies could be seen in the P.M.C. of the resting stage. It is, however, worthy of note that these bodies persist no further. In this respect the hybrid seems to resemble the *prostratum* parent for, in *orientale*, these bodies persist right up to the end. It seems probable that persistence of the nucleolus is a Mendelian recessive. The hybrid shows in some characters, resemblance to the *prostratum* parent a Mendelian dominance. Non-persistence would appear to be dominant to persistence. Hence we find the hybrid showing non-persistence. Full details regarding inheritance of characters by the hybrid are given in a separate paper.

During diakinesis only a few chromosomes pair while the others remain as univalents. The bivalents and the univalents are arranged peripherally around the nucleolus (Fig 33). The most frequent number of bivalents met with based on an examination of a large number of pollen mother cells is eight.

After diakinesis, the prophase stage sets in when the bivalents and univalents appear clumped at the centre of the cell (Fig 34). The nuclear membrane disappears at this stage and along with it the nucleolus. This stage comes to an end when the spindle fibres make their appearance.

During Metaphase I the chromosomes separate and unlike in normal meiosis, the chromosomes fail to arrange on the equatorial plate. The bivalents and the univalents are scattered on the spindle. The most frequent arrangement is for the bivalents to occur at the equator and for



TEXT FIGS 28-47—Figs 28 31 *Meiosis in Sesamum prostratum* Fig 28 Somatic plate of *Sesamum prostratum* showing 32 chromosomes Fig 29 Three tapetal cells showing mitotic division  $\times 400$  Fig 30 Resting nucleus of the P M C showing nucleolar budding in various degrees  $\times 400$  Fig 31 Metaphase I polar view showing 16 bivalents Figs 32-47 *Meiosis in the sterile hybrid* Fig 32 Somatic complement showing 29 chromosomes Fig 33 Diakinesis showing bivalents and univalents Fig 34 Prometaphase showing clumped chromosomes Figs 35 38 Metaphase I showing bivalents and the univalents scattered The bivalents are at the equatorial region while the univalents are nearer the poles Fig 39 Metaphase I showing 8 bivalents 1 trivalent and 10 univalents Fig 40 Metaphase I showing 9 bivalents, 1 trivalent and the rest univalents Fig 41 Anaphase I Irregular disjunction of the chromosomes Figs 42 and 43 Univalents and bivalents lagging in the spindle during anaphase Fig 44 Formation of chromosome bridges during anaphase I Fig 45 Interphase nuclei showing two cells having unequal number of chromosomes with the left out univalents in the cytoplasm of the P M C Fig 46 Metaphase II with unequal number of chromosomes Fig 47 Anaphase II with the usual lagging chromosomes All figures have been drawn at a magnification of  $\times 3000$  unless otherwise stated

the univalents to be scattered at the poles (Figs 35, 36, 37 and 38) Fig 39 shows 8 bivalents, 1 trivalent and 10 univalents Fig 40 shows the metaphase side-view representing 9 bivalents, 1 trivalent and the rest univalents

There seems to be some relationship between the degree of synapsis and the arrangement of chromosomes in the equatorial region In all cases where weak pairing is exhibited by the chromosomes this scattered condition prevails Many cases of haploidy have been cited to show that asynapsis and absence of a regular equatorial plate at Metaphase I, go together Haplonts of *Nicotiana Tabacum* (Chipman and Goodspeed, 1927) and *Nicotiana glutinosa* (Goodspeed and Avery, 1929) were observed to show this feature Catcheside (1932) recorded such a behaviour in a haploid *Oenothera* and states in that connection that "many of the chromosomes have never been at the equator of the spindle, but have a definite bias towards one or the other end of the poles ever since diakinesis" Humphry (1934) has reported such cases in haploid tomatoes Many examples of interspecific hybrids in the genus *Nicotiana* may be cited to show this prevailing condition of scattered arrangement of the chromosomes, *Nicotiana sylvestris*  $\times$  *Nicotiana tomentosa* (Goodspeed and Clausen, 1928), *Nicotiana bigelovii*  $\times$  *Nicotiana solanifolia* and *Nicotiana Tabacum*  $\times$  *Nicotiana rustica* (Goodspeed, 1934), *Nicotiana glutinosa*  $\times$  *Nicotiana Tabacum* (Raghavan and Srinivasan, A R, 1941 a)

Thus during first metaphase stage the chromosomes are scattered along the whole length of the pollen mother cell Their weak pairing during diakinesis and the consequent scattered arrangement of the chromosomes during metaphase constitute cytological basis for the sterility of the hybrid

The metaphase stage which is characterised by random distribution of the bivalents and the univalents is followed by anaphase which is equally irregular. In normal pollen mother cell, anaphase is characterised by uniform disjunction of the bivalents which results in an equal distribution of chromosomes. But in the case of the hybrid, the bivalents and the univalents during their disjunction exhibit various irregularities.

Fig. 41 shows the migration of the bivalents and the univalents to the poles. Some univalents are seen left out of the spindle and they seem to divide. These divided bits of univalents either reach the poles along with the separating bivalents or they are left out in the cytoplasm where they remain to the last without being included in any of the daughter nuclei.

Frequently bivalents and univalents are seen to lag on a spindle (Figs. 42 and 43). These laggards also get included in one of the daughter nuclei or they remain in the cytoplasm during the interphase stage. These organize themselves into groups and finally form a membrane around them to form the micronuclei (Fig. 50). Sometimes they are found in the plasma in the succeeding stages. In some cases they are included in one of the daughter nuclei. Similar cases of laggards have been recorded in many hybrids. In *Nicotiana* hybrids, *N. glutinosa*  $\times$  *N. Tabacum* (Raghavan and Srinivasan, A. R., 1941 a), in *Brassica* hybrids (Morinaga, 1929) (Ramanujam, 1943), laggards of a similar kind have been found frequently.

Sometimes due to unequal disjunction, the separating chromosomes are connected by long chromatin thread, forming chromatin bridges (Fig. 44). The exact nature of these bridges and the reason for their formation could not be studied in detail.

After the complete separation of the chromosomes, interphase sets in. The two chromosome groups organize into two nuclei at the poles. It is observed that one of the poles contains a larger number of chromosomes than the opposite pole (Fig. 45). This is due to the unequal separation that takes place during anaphase. Further some of the chromosomes have been left out as laggards in the plasma itself. Hence the disparity in number of chromosomes between the two interphase nuclei. No wall is formed between them. Wall formation after the first division is not a common feature of the dicotyledons. But in *Nicotiana* hybrids, between *Nicotiana glutinosa* and *Nicotiana Tabacum* (Raghavan and Srinivasan, A. R., 1941 a), wall formation has been recorded.

Second metaphase plate shows two groups of chromosomes distributed with unequal numbers. Some of the laggards are also seen in the cytoplasm. These laggards remain as such and are not included in the second metaphase plate (Fig. 46).

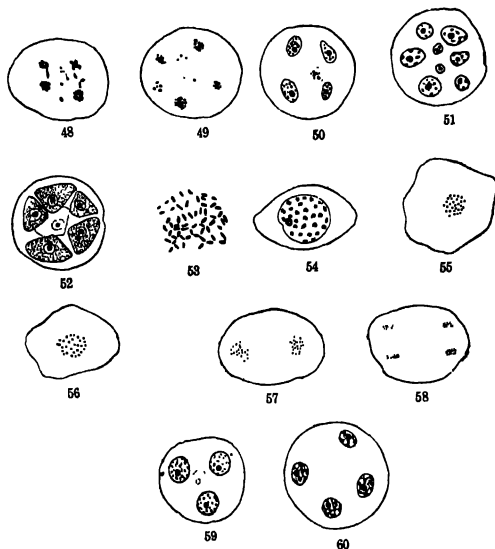
During the succeeding second Anaphase also the chromosomes separate exhibiting irregularities of the kind occurring during first anaphase (Fig 47) Some chromosomes are left behind on the spindle Some remain out of the spindle and assemble together As in first anaphase equal number of chromosomes do not go to each pole As a result of these groups having varying numbers of chromosomes are formed and the four daughter nuclei are organised around each one of these groups (Figs 48 and 49) A few laggards are also seen in the cytoplasm which are not included in any of the telophase nuclei The discrepancy in the number of chromosomes in the telophase groups is responsible for the formation of the daughter cells of unequal size which ultimately results in tetrads and pentads, big and small Fig 51 shows daughter cells of unequal size being formed Since the chromosomes of the haploid complement are not evenly distributed among the four telophase nuclei, the grains that result from them are non-viable, leading to the sterility of the hybrid In some cases, some of the laggards that were left out of the spindle arrange themselves in small groups and form micronuclei (Fig 50) Thus instead of regular tetrads being formed, pentads and hexads result out of the irregularities of meiosis in the hybrids (Figs 51 and 52) Hence grains exhibit various sizes and shapes resulting in their polymorphic nature Plate XII, Fig 3 is a microphotograph of the pollen grains of the sterile hybrid, to show the polymorphic grains Only 5 to 10% of the pollen grains reached the size of the pollen grains of the parents

The pollen grains are highly non viable They do not germinate even in sugar agar cultures The pollen grains were deposited on the stigma of both the parents, *Sesamum orientale* and *Sesamum prostratum* In both the cases, the grains did not germinate Pollen grains were deposited on the stigma of the sterile hybrid itself Even then the result proved negative

Thus it is evident from the above observations that the sterility of the hybrid is the outcome of the irregularity of meiosis

#### (d) The Amphidiploid

Weak pairing between the parental chromosomes in the hybrid indicates that there is no homology between the chromosomes of *Sesamum orientale* and *Sesamum prostratum* Presumably the parental chromosome sets exhibit structural disparity, hence there is little possibility of their coming together in pairs This absence of pairing due to the weak homology between the chromosomes could be overcome by supplying these two sets of chromosome complements, each with another homologous set Thus 13 *orientale* chromosomes may be supplied with another 13 of its own so that both the



TEXT-FIGS. 48-60.—Figs. 48-52 *Meiosis of the sterile hybrid*. Figs. 48 and 49, Anaphase groups of chromosomes containing varied number of chromosomes and also a few left out laggards. Fig. 50, Formation of 4 telophase nuclei and organisation of the micronucleus by the lagging chromosomes. Fig. 51, P.M.C containing 8 telophase nuclei of different sizes. Fig. 52, P.M.C. containing 6 daughter cells as a result of irregular meiosis. Figs. 53-60 *Meiosis in the Fertile Hybrid (the amphidiploid)*: Fig. 53 Somatic complement showing 58 chromosomes. Fig. 54, Diakinesis showing 29 bivalents. Figs 55 and 56, Metaphase I showing 29 bivalents at the equator. Fig. 57, Metaphase II showing the equatorial plates containing 29 chromosomes each. Fig. 58, Anaphase II showing normal disjunction. Fig. 59 Telophase nuclei arranged three in one plane and one below. Fig. 60, Telophase nuclei, all the four in one plane. All figures have been drawn at a magnification of Ca. 3,000 unless otherwise stated.

sets may pair among themselves. Similarly for *Sesamum prostratum* another set of 16 chromosomes may be made available so that pairing may take place between the two sets of 16 chromosomes. This has been made possible recently by colchicine application by which doubling of genes is brought about.

In the present investigation the 29 chromosomes of the sterile hybrid have been doubled ( $2n$  58). That is, the 13 chromosomes of *orientale* have been duplicated as also the 16 chromosomes of *prostratum*. The result is, the hybrid proves to be completely fertile unlike its sterile predecessor. The cytological explanation of the fertility is the regularity with which meiosis takes place.

Fig 53 shows a somatic plate containing 58 chromosomes which is double the number of the sterile hybrid ( $2n$  29). But a distinction could not be made between the two parental sets of chromosomes since they were identical in their morphology.

**Meiosis**—Microsporangial development is of the normal type as described for *Sesamum orientale*.

Meiosis is regular. At diakinesis there is regular pairing and 29 bivalents are formed (Fig 54). Metaphase I (Figs 55 and 56) shows the 29 bivalents arranged in the form of a flat plate in the equator. Obviously pairing has taken place among the duplicated parental genomes, that is, 16 *prostratum* with 16 *prostratum* and 13 *orientale* with 13 *orientale* chromosomes.

First Anaphase is normal and the chromosomes disjoin without exhibiting any irregularity. Fig 57 shows the metaphase plates during Metaphase II. Second anaphase also is regular (Fig 58). Equal numbers of chromosomes go to the respective poles. As a result of regular meiosis, the four telophase nuclei are organised with equal number of chromosomes and tetrads are organised in the normal way (Figs 59 and 60).

The pollen grains that are formed are of uniform shape and size without showing any polymorphism. They are however bigger in size than those of the parents. Plate XII, Fig 4, is a microphotograph of the pollen grains of the fertile hybrid.

The pollen grains are highly viable as evidenced by the formation of a large number of fruits in the fertile hybrid. Also experiments on germination of the pollen grains in agar culture have shown the rapidity with which the grains germinate. Consequently the fertility of the hybrid has increased the yield of an individual plant by 6 times its parent, *Sesamum orientale*.

The ovary is fertile as revealed by the presence of a large number of seeds which in each fruit amounts to about 50. The amphidiploid thus derived is breeding true and the meiosis in all the subsequent generations have been found to be quite regular. This fertile hybrid may be regarded as a stable true-breeding species, deserving an independent position along with the parents, *Sesamum orientale* and *Sesamum prostratum*.

#### IV. DISCUSSION

##### (a) Nucleolus—its behaviour and persistence

In angiosperms normally the nucleolus appears at the telophase stage and remains till the onset of metaphase, after which it disappears along with the disappearance of the nuclear membrane. However, cases where the nucleolus persists even after the metaphase stage are not uncommon. In *Polanisia trachysperma* persistence upto metaphase in somatic mitosis was recorded by Raghavan (1938). But the nucleolar persistence throughout meiotic stages such as was seen in *Sesamum orientale* is comparatively rare. The behaviour of these nucleolar bodies is varied.

The persistent nucleolus arises as a spherical bud from the nucleolus of the resting nucleus. Many such buds are formed, most of them disappearing during diakinesis stage except one which persists with the same size neither diminishing nor dividing until finally incorporated in the tetrad cells. There are also cases where this body after remaining for most of the stages of meiosis, disappears into the cytoplasm.

The possibility of these bodies being chromosomes or their fragments is ruled off by the fact that they are bigger than the chromosomes and perfectly spherical in shape. They take the stain to the same extent as the nucleolus does. During meiotic stages they do not play any part. Thus it may be seen that by their origin from the margin of the big nucleolus and by their taking up the stain to the same extent, these bodies are nucleolar in nature.

The persistent nucleolus in the pollen mother cells of *Sesamum orientale* is observed to exhibit varied movements. During the earlier stages of meiosis it either remains in the equator or lies apart. If it remains in the equator it migrates to the pole when anaphase separation sets in; or in some cases it disappears altogether. If they persist they get incorporated into one of the tetrad cells. Thus it stands to reason that some force has been acting upon these persistent nucleolar bodies to enable them to execute such movements.

The factors which are responsible for such a movement have not been clearly established. They are presumably acted upon by the same forces

which are responsible for chromosome movements, like the contraction of spindle fibres, electromagnetic repulsion or attraction set up by cytoplasmic currents in the spindle region. Mensinkai (1939) regards the division and migration of the persistent nucleolus as being due to the stretching of these spindle fibres. But since the persistent nucleolus has been found to have no connection with the spindle fibre, it is unlikely that the contraction of the spindle brings about the movement of the nucleolus. The other alternative is the magnetic attraction set up by the cytoplasmic current. The fact that sometimes the persistent nucleolus remains at the equator while at other times it moves towards the poles would suggest that these movements may be due to the above cause. Further the spindle region would appear to be one of localised forces and when a body lies in that region it is carried away provided it is not attached to any thing like the spindle fibre. The persistent nucleolus by its position at the border of the spindle fibres and also by its migration to the poles ahead of the chromosomes, it would appear that the movement of the nucleolus might be controlled by the localised forces that have been referred to above.

During metaphase the chromosomes are attached to the spindle fibres and since contraction of the fibres takes place only a little later, the chromosomes are prevented from being carried away by the forces at the spindle area, while the nucleolus lies unattached to the spindle fibres so that it is free to move and hence it is found to reach the poles ahead of the chromosomes. Movements of this kind may perhaps be explained by the electromagnetic theory of nuclear division (Kuwada and Sugimoto, 1928). According to this theory, the persistent nucleolus, because of the presence of plastin, which it has retained instead of giving to the chromatin, becomes highly electropositive, while the poles remain oppositely charged. As a result of this the persistent nucleolus is attracted towards the pole and hence the movement. It is further explained that in the normal cases where there is no persistent nucleolus, the chromosomes change their electric charge due to the transference of the plastin from the nucleolus. A similar explanation for the differential movement of the persistent nucleoli and the chromosomes would seem probable in the present instance also.

(b) *Interspecific hybridisation—a guide to ancestral homology.*

Interspecific and intergeneric hybridisations and the study of the behaviour of such hybrids have long been engaging the attention of many cytologists, since the results of the observation serve as valuable clues to determine the relationship between the various species. The mode of origin of new species can be inferred with the help of hybridisation results as disclosed by cytological data.

Species having the same number of chromosomes when crossed with each other will give either fertile hybrids or hybrids of partial or completely sterile nature. Such fertile hybrids are met with in the following cases: *Viola* (Clausen, 1931), *Nicotiana* (Goodspeed, 1934) and *Triticum* (Aase, 1930). Clausen (1931) crossed *Viola tricolour* ( $n=13$ ) with *Viola alpestris* ( $n=13$ ) and got a hybrid which was fertile ( $2n=26$ ). During meiosis, he observed that 13 chromosomes of *Viola alpestris* paired completely with 13 chromosomes of *Viola tricolour* thus resulting in a fertile hybrid. The complete synapsis in this case indicates the complete homology of the two sets of chromosomes. Hence these two species, though taxonomically distinct, may be regarded as having had a common origin on the basis of this piece of cytological evidence.

In the case of sterile hybrids, some show partial pairing with varying number of bivalents and univalents during meiosis. In such cases the greater the number of bivalents formed, the greater has been the fertility of the hybrids. For instance, Clausen (1931) crossed *Viola nana* ( $n=24$ ) with *Viola lutea* ( $n=24$ ). The hybrid was found to be partially fertile; meiotic stages showed the presence of only a few bivalents, 6 to 8. In the normal case if there is complete pairing there should be 24 bivalents formed. But as there were only a few bivalents, the hybrid was partially sterile, correspondingly in the hybrids between *Viola orphnuds* ( $n=11$ ) and *Viola cornuta alba* ( $n=11$ ) he found a greater number of bivalents amounting to 9 or 10. The hybrid was almost completely fertile. Thus it would appear that the degree of hybrid fertility is directly proportional to the number of bivalents formed during the meiotic stages of the hybrids.

In the case of some hybrids, pairing is totally absent and consequently the hybrids are completely sterile. Karpechenko (1927 a, 1927 b) got a hybrid which was completely sterile, by an intergeneric cross between *Raphanus sativus* ( $n=9$ ) and *Brassica oleracea* ( $n=9$ ). This complete sterility was attributed to the total absence of synapsis or pairing of parental chromosomes during meiosis. This only confirms the previous inference that the degree of synapsis is a measure of the degree of hybrid fertility.

Thus it is noticeable that though the two parental chromosomes of hybrids are equal in number, yet they vary in their degree of affinity indicating thereby that the pairing of chromosomes does not depend upon the numerical identity of the chromosomes but on their structural and morphological homology. This homology between chromosomes of two gametic sets will be nearer if both the parents have had a common origin. Thus the cytological behaviour of species hybrid indicates not only the extent of homology between the species but also the ancestry of the parental forms.

In the case of hybrids derived out of parents having different chromosome numbers, the behaviour of hybrids exhibits complication which is nonetheless interesting

The behaviour and the extent of affinity of the chromosomes in such hybrids as disclosed by their behaviour at meiosis has been classified by Tackholm (1922) into three groups. They are (1) *Drosera* scheme of pairing where there is strong affinity between parental chromosomes, (2) *Hieracium Boreale* type where there is a weak affinity and (3) the *Pygraea* type where there is no affinity. It was Rosenberg (1909) who first observed this phenomenon of pairing in *Drosera* hybrids. He crossed *Drosera rotundifolia* ( $2n = 20$ ) with *Drosera longifolia* ( $2n = 40$ ). As a result he got a hybrid containing 30 chromosomes, 10 from the *rotundifolia* parent and 20 from the *longifolia* parent. During synapsis only 10 bivalents were formed and 10 chromosomes remained as univalents. Rosenberg concluded that the 10 chromosomes of *rotundifolia* paired with 10 of *longifolia* leaving the other 10 of *longifolia* unpaired. Such a type of pairing between two sets of chromosomes belonging to two different parental species which may or may not have equal number of chromosomes is known as Allosyndesis. Here 10 chromosomes of *rotundifolia* and 10 chromosomes of *longifolia* paired allosyndetically. Similar cases of allosyndesis have been recorded in *Triticum* hybrids ( $n = 35$ ) resulting from a cross between *Triticum Emmer* ( $n = 14$ ) and *Triticum Vulgare* ( $n = 21$ ). 14 synaptic pairs were formed. 14 chromosomes of *Triticum Emmer* paired with 14 of *Triticum Vulgare* while the remaining 7 chromosomes of *Vulgare* parent were left in an unpaired condition (Kihara, 1919; Sax, 1922). In *Nicotiana* hybrids between *Nicotiana Tabacum* ( $n = 12$ ) and *Nicotiana sylvestris* ( $n = 24$ ) (Goodspeed and Clausen, 1927) there was an arrangement of 12 bivalents and 12 univalents indicating allosyndesis between 12 chromosomes of *Nicotiana Tabacum* and 12 chromosomes of *Nicotiana sylvestris* ( $n = 24$ ).

There are also cases where in addition to allosyndesis there is also autosyndesis. Autosyndesis indicates the pairing among the chromosomes of a single set. Thus in *Digitalis* hybrids ( $2n = 72$ ), between *D. lutea* ( $n = 48$ ) and *D. micrantha* ( $n = 24$ ) 72 chromosomes were found in the somatic complement, and during meiosis they organised into 36 gemini (Haase-Bessel, 1916) indicating that all the chromosomes have paired. It means that 24 chromosomes of *D. micrantha* have paired with 24 chromosomes of *D. lutea* to form 24 bivalents. The remaining 24 chromosomes of *D. lutea* have paired among themselves to form 12 more bivalents, thus bringing the total to 36 bivalents. In such a case as this, there is not only pairing between the members of the gametic complements of the two different species, namely,

*D. lutea* and *D. micrantha* (allosyndesis) but also among the remaining chromosomes of the same gametic complement, namely *D. lutea* (autosyndesis).

Similarly in *Papaver* hybrids (Ljundahl, 1924), viz., *Papaver nudicaule* ( $n:7$ ) and *Papaver radicum* ( $n:35$ ) there are 21 gemini formed which may be explained on the same basis. 7 chromosomes of *nudicaule* have paired with 7 of *radicum* and the 28 chromosomes of *radicum* have paired among themselves to form 14 gemini. Thus there is allosyndesis between 7 of *radicum* and 7 of *nudicaule* and autosyndesis between the 28 chromosomes of *radicum* themselves. This revealed that though the two parental complements differ in number yet there is a marked affinity between the two sets. Allosyndesis and Autosyndesis would thus indicate the extent to which there exists homology between the two parents.

In hybrids exhibiting weak pairing among the chromosomes of the parents, varying degrees of synapsis and in some cases asynapsis also occur in the meiotic cycle of the hybrid and consequently it becomes sterile. Raghavan and Srinivasan, A. R. (1941 a) record such weak pairing among the chromosomes in the hybrids between *Nicotiana glutinosa* ( $2n:24$ ) and *Nicotiana Tabacum* ( $2n:48$ ). They observed varying degrees of synapsis and also in certain cases complete asynapsis. This would indicate distant homology. This hybrid has been classified by them under the *Hieracium Boreale* type. Hybrids belonging to the last scheme, namely, showing no affinity between the members of the two complements have been recorded in many cases. *Crepis* (Collins and Mann, 1923), *Digitalis* (Haase-Bessel, 1921) and *Nicotiana* (Goodspeed, 1934).

In the present investigation the meiosis of the sterile hybrid was studied in detail with a view to find out the degree of affinity that existed between the two sets of gametic complements. The sterile hybrid ( $2n:29$ ) of the cross between *Sesamum orientale* ( $n:13$ ) and *Sesamum prostratum* ( $n:16$ ) shows irregular meiosis with varying numbers of bivalents. It is also found that in the majority of cases 8 bivalents are formed with the rest scattered as univalents. The somatic number 29 of the hybrid should contain 13 of *orientale* chromosomes and 16 of the *prostratum* parent. If it conforms to the *Drosera* scheme of pairing, then 13 of *orientale* chromosomes should pair with 13 of *prostratum*, leaving the three *prostratum* chromosomes unpaired. But such a maximum pairing has never been observed to take place as most of the pollen mother cells show 8 and very occasionally 10 bivalents. This can be interpreted in two ways: (1) That the 8 chromosomes of *orientale* pair with a corresponding number of *prostratum* chromosomes leaving the others

unpaired. This means that it is a case of allosyndetic pairing. (2) That the *prostratum* chromosomes might pair among themselves to form 8 bivalents, leaving the 13 *orientale* chromosomes in an unpaired condition. This suggests autosyndesis among *prostratum* chromosomes. Either of these interpretations would indicate only a weak homology between the chromosomes of *Sesamum prostratum* and *Sesamum orientale*. It is therefore reasonable to infer that we have to look to some other source for the origin of the cultivated til (*Sesamum orientale*) than from *prostratum*. It is not likely that they could have had a common origin on account of their distant homology as revealed by the behaviour of their chromosomes in the hybrid. This would appear to be supported also by the fact that even though they belong to the same genus, they are different in their habit. The one is erect whereas the other is prostrate. *Sesamum orientale* is an annual herb whereas *Sesamum prostratum* is perennial almost a shrub. It may be that future explorations into the Indian wilds may show the presence of ancestral forms of the domesticated til. In this connection, we have also to remember the American tropics. Only one wild species has been reported from Argentina, which is *Sesamum radiatum* ( $2n : 64$ ) (John and Rao, 1941). Its number suggests tetraploidy from *Sesamum prostratum*. Whether there are any more forms which could throw light upon the origin of the cultivated til, future exploration alone can reveal.

(c) *Artificial synthesis of a new species*

The genes on the chromosomes govern plant characters. Any alteration of the genes either in their position or in their number would consequently affect the configuration of a plant. Gene mutations thus bring about mutations of plant characters. Generally gene mutations involve a rearrangement of the genes such as inversion, reciprocal translocation, etc. These lead to mitotic and meiotic aberrations resulting in external morphological mutations of several kinds. A more common and fruitful way in which changes in plant configurations occur is through the duplication of genes of certain chromosome sets or the duplication of the entire genic complement. That is, all the members of the chromosome complement undergo reduplication and this is known as Polyploidy. The phenomenon of polyploidy may be of two kinds, Autopolyploidy and Allopolyploidy. In the former there is a duplication of the chromosomes derived from the same parent (as in self-pollinated plants) or from parents belonging to the same species as in cross-pollinated plants. In the latter two sets of chromosomes from two different parents are involved. This may happen in interspecific hybrids and very rarely in intergeneric hybrids,

Autopolyploids arise either spontaneously in nature or are artificially induced. Allopolyploidy on the other hand indicates hybridisation, whether interspecific or intergeneric. Autopolyploids may be stable species breeding true. In most cases where there is induced polyploidy sterility of the autopolyploid is quite common as in *Cosmos* (Earl Newcomer, 1941). Allopolyploids as aforesaid arise out of hybridisation. The hybrid so derived may be sterile or fertile. If the hybrids prove fertile, then the allopolyploids breed true and establish themselves as stable species. Allopolyploids which are sterile due to hybridisation may be made fertile by artificial induction of amphidiploidy about which a detailed mention is made further below.

The most common form of autopolyploids occurring in nature are the tetraploids. Tetraploids arise as a result of the duplication of the diploid chromosomal set. Many causes are in evidence for the duplication of chromosomes in the plant cell. Cytomyxis, occurring in the pollen mother cells, is considered by some to be one among them. This phenomenon was first observed by Gates (1911) in the pollen mother cells of *Oenothera gigas*. He described the process as a migration of the chromatic material from the one pollen mother cell into the adjacent cell. But he contended that the chromatic material disappeared into the cytoplasm of the recipient cell and that the chromatic material of the recipient cell was not increased by the addition of extruded chromatic material from the adjacent cell. Thus according to him, cytomyxis does not bring about chromosome duplication. Binucleate pollen mother cells arisen from cytomyxis, have been recorded in *Tridax* (Raghavan and Venkatasubban, 1941) where the two nuclei enter independently into successive division stages and ultimately it was observed that this phenomenon was responsible for the degeneration of the pollen mother cells and the significant sterility in the species was attributed to cytomyxis. Nandi (1937) also describes such cases of binucleate pollen mother cell formation from cytomyxis and diakinesis in *Oryza*. Particularly in the case of hybrids both interspecific and intergeneric this abnormal phenomenon seems to be quite common. Kattermann (1933) in *Triticum* × *Secale* hybrids, Percival (1930) in hybrids between *Aegilops* × *Triticum* species and Raghavan and Srinivasan, A. R. (1941 a) in *Nicotiana glutinosa* × *Nicotiana Tabacum* hybrids. From this and from the evidence of Church (1929) who found the occurrence of this phenomenon in the hybrids of *Phalaris*, we may infer that it is more probable that this phenomenon is associated with hybrids than being an artifact as Sinoto (1922) regarded. It cannot however be said with any amount of certainty whether the formation of additional nuclei through cytomyxis is a certain method of origin of polyploidy.

Tetraploidy may also arise from fusion of unreduced gametes having the diploid number of chromosomes. They are formed due to absence of cross-wall formation after the heterotypic division. When an unreduced gamete fertilises a gamete with the haploid number, then a triploid results. If it fertilises another unreduced gamete, a tetraploid is formed. Such instances of tetraploidy are common in *Datura*, and Tobacco. In *Brassica* hybrids it was observed by Ramanujam and Srinivasachar (1943).

Experimental tetraploids have been obtained in a number of species. As early as 1914 Gregory described a tetraploid strain in *Primula sinensis* containing 48 chromosomes. It has been shown that in the chromosome sets of diploids there are chromosomes of different kinds, each of which is represented twice, one of the two being derived from egg and the other from the pollen. In the tetraploids with 48 chromosomes, it was found that the chromosomes often came together in fours at reduction division. It was found that the 48 chromosomes of the tetraploid unted into 12 groups of four. Winkler (1916) induced polyploidy in *Solanum* by grafting together the species *Solanum lycopersicum* and *Solanum nigrum*. The adventitious shoots arose at the grafting point were in some cases tetraploid. Decapitation is another means of bringing about tetraploidy. Terminal buds of tomato, tobacco have been decapitated and callus allowed to form which produced adventitious buds from which arose tetraploids (Beadle, 1940).

Certain drugs, particularly the alkaloid, Colchicine extracted from *Colchicum autumnale* have been known to produce characteristic disturbances in the cell division. Blakeslee and Avery (1937) showed that treating seeds with an appropriate solution of colchicine produces tetraploid tissues from which tetraploid strains may be derived. Their work has been subsequently confirmed and employed by many investigators (Nebel and Ruttle, 1938), (Levan, 1938, 1939 and 1940 a) and many others.

An important property of Autopolyploids concerns the behaviour of their chromosomes at meiosis. In a diploid organism, every chromosome has its homologous partner. Of the two homologous sets one is from the male cell and the other belongs to the female sexual cell. A number of bivalents equal to the haploid chromosome number is formed and the disjunction at meiosis gives rise to gametes all of which contain haploid sets of the chromosomes. In an autopolyploid every chromosome has more than one homologue so that opportunity presents itself for the formation of trivalents, quadrivalents and higher associations. The disjunction at meiosis is frequently abnormal, different numbers of chromosomes going to two poles of the division spindle. In order to breed true, an autotetraploid

must produce gametes all of which have the same complements of chromosomes. Since loss or addition of chromosomes usually reduces the viability of the offspring, the reproductive cells of polyploids are frequently non-functional. But there are a number of cases of tetraploids which are normal and consequently breed true and have established themselves as stable forms, e.g., *Tradescantia* (Anderson and Sax, 1936).

Apart from their existence in nature, due to natural hybridisation, allopolyploids have been produced experimentally. The intergeneric hybrids between radish (*Raphanus sativus*,  $n:9$ ) and cabbage (*Brassica oleracea*,  $n:9$ ) serve as an illustration of the results obtained when the chromosome complement is reduplicated in crosses of taxonomically remote forms (Karpechenko, 1927 *a* and *b*). Both parents have the diploid number 18. Cross succeeded fairly easily. The hybrids had 18 chromosomes, 9 from the radish and 9 from the cabbage parent. No chromosome pairing took place and the 18 chromosomes remained as univalents at metaphase of the first division and were distributed at random to the poles. At the second division the univalents split, giving rise to cells with a varying number of chromosomes, mostly from 6 to 12. In some of the pollen mother cells however, the first division was abortive and nuclei were formed that included all the 18 univalents. The second division then gave rise to two diploid spores. Two pollen grains containing the diploid complements were organised. The  $F_1$  hybrids mostly were sterile but few seeds were produced. Cytological examination showed that most of the  $F_2$  hybrids derived from the seeds had 36 chromosomes in their somatic cell. The origin of such plants was in all probability due to the union of the few diploid gametes produced in the  $F_1$  hybrid. The  $F_2$  plants therefore contained 18 radish and 18 cabbage chromosomes; in other words, the diploid complement of the chromosomes of each parental species. Such  $F_2$  hybrids were allotetraploids. The meiotic divisions were very regular in striking contrast with the abnormalities observed at meiosis in the  $F_1$  hybrids. In the tetraploids, 18 bivalents were formed, disjunction was normal and the resulting cells contained 18 chromosomes each. It is practically certain that 18 bivalents that appeared at meiosis were due to the pairing of 9 radish chromosomes with their 9 radish homologues. Thus the pairing was between similar chromosomes of the same parent (Autosyndesis) rather than between the chromosomes of different species (Allosyndesis). The tetraploid plants were fertile and bred true. This true breeding type was assigned the name *Raphano-Brassica* because it arose out of the two genera, *Raphanus* and *Brassica*, after hybridisation.

*Raphano-Brassica* is by no means the only new species which has arisen through allopolyploidy in experimental cultures. *Primula Kewensis* ( $n:18$

and  $2n:36$ ) is another allotetraploid which arose as a bud sport among population of *Primula floribunda* and *Primula verticillata*, both having haploid number 9. *Primula Kewensis*, the diploid hybrid of *Primula floribunda* and *Primula verticillata*, was observed to set seed on three occasions since its first production in 1900 (Newton and Pellew, 1929). Each time its seed gave rise to fertile plants with the tetraploid number of chromosomes,  $2n:36$ . In the vegetative cells of one of the fertile inflorescences, tetraploid number of chromosomes was found, showing that the doubling process took place in the somatic division. It was the only case known of a sterile (diploid) hybrid giving rise to a fertile tetraploid by somatic doubling of chromosomes. In the meiotic division of the diploid hybrid of *P. Kewensis* ( $n:18$ ) 9 pairs of chromosomes were formed which may be indicated as F1 V1, F2 V2 and so on. The resulting gamete would contain all possible combinations of chromosomes. Most of these gametes were non-viable; a few however were viable and these while they bore many *P. floribunda* characters also showed traces of *P. verticillata*. But in the tetraploid hybrid each chromosome was represented twice and if 18 pairs were formed in meiosis, they might either be pairs of identical chromosomes (F1 F1, V1 V1) or of corresponding *floribunda* and *verticillata* chromosomes (interspecific pairing) as in the diploid hybrid. In the last case, the number of possible combinations would be much greater than in the diploid. In the former case, F1 F1, V1 V1 or identical chromosomes separate and the gamete will each contain a complete set of *floribunda* or *verticillata* chromosomes which on fertilisation will give a uniform progeny. Thus the hypothesis of pairing of identical chromosomes (intraspecific) gives a satisfactory explanation of a perfectly constant tetraploid hybrid. This hypothesis was put forward by Winge (1917) in discussing the possible origin of tetraploids from hybrids. He considered that doubling of chromosomes might result in failure to conjugate at meiosis, followed by splitting and subsequent pairing of the identical halves.

The case of *Crepis* is somewhat different from that of *Primula Kewensis*. Poole (1931) showed that in the diploid hybrid of *Crepis*, *Crepis rubra* ( $n:5$ )  $\times$  *Crepis fetida* ( $n:5$ ) there was complete pairing of the chromosomes. They behaved as though they were from the same parents. Consequently, the hybrid was fertile and the tetraploid derived from it behaved almost like an autotetraploid. Quadrivalent formation was very common. In the  $F_1$  hybrid R (*rubra*) and F (*fetida*) chromosomes paired (RF). In the tetraploid form duplication of the chromosomes took place, resulting in RR and FF. Because of the complete homology of R and F chromosomes, these four chromosomes formed one quadrivalent (RRFF). But in the case of

*Primula Kewensis* tetraploid (FFVV) there were no quadrivalents formed. Instead F and F paired and V and V paired forming 18 bivalents. It might be that even though F and V were somewhat related, they were not completely homologous so as to induce quadrivalent formation. Presumably VV and FF bivalents may exhibit secondary association indicating their ancestral relationship.

Experimentally-produced allopolyploids of the kind described above happen to be identical to wild Linnæan species already existing in nature. The classical example of such an allotetraploid is that of *Galeopsis Tetrahit*, an existing Linnæan species which was experimentally synthesised from its putative ancestors. In his monograph on the genus *Galeopsis*, Muntzing (1920, 1932 and 1937) showed that six out of the eight species investigated had the haploid number of chromosomes 8 and the two remaining ones had  $n = 16$ . Among the former were the species of *Galeopsis pubescens* ( $n = 8$ ) and *Galeopsis speciosa* ( $n = 8$ ) and among the latter was *Galeopsis Tetrahit* ( $n = 16$ ). The crosses between *G. pubescens* and *G. speciosa* succeeded easily when *G. pubescens* was used as the female parent. At meiosis varying numbers of bivalents and univalents were formed. The anther of the flowers of this hybrid contained only 8 to 20% of good pollen grains. A few good ovules however were produced. In the  $F_2$  progeny raised by the few seeds obtained, a single plant was found that proved to be a triploid ( $3n = 24$ ). Its origin is probably due to the union of a gamete containing the somatic complement of the hybrid (8 chromosomes of *G. pubescens* and 8 chromosomes of *G. speciosa*) with a gamete carrying 8 chromosomes. This triploid was backcrossed to pure *G. pubescens*. A single seed resulted from the backcross. It gave rise to a plant which proved to be a tetraploid ( $4n = 32$ ). This tetraploid was fertile and became the progenitor of a strain which was named "artificial Tetrahit".

This 'artificial Tetrahit' was like the real *Galeopsis Tetrahit* described by Linnæus in possessing 32 chromosomes in somatic cells and 16 bivalents at meiosis. The irregular meiosis characteristic of the  $F_1$  hybrids ceased to exist in the artificial Tetrahit. In short, the artificial *G. Tetrahit* and the natural species are similar not only in their morphology but also in their genetical and cytological behaviour.

*Spartina Townsendii* ( $2n = 56$ ) is another example of an experimental allotetraploid. *Spartina stricta* ( $n = 28$ ) and *Spartina alternifolia* ( $n = 28$ ) were crossed (Huskins, 1931). The tetraploid form of the hybrid was found to contain 112 chromosomes. *Spartina Townsendii* showed a diploid number of 56 chromosomes and with morphological and cytological evidences,

Huskins proved that *Spartina Townsendii* was an allotetraploid derivative of the hybrid between *Spartina stricta* and *Spartina alternifolia*

Since the discovery of colchicine as an agent for the doubling of chromosomes, several experiments have been conducted to confirm the origin of existing species by artificially repeating the supposed event that led up to their formation. Thus existing polyploid species have been artificially synthesised from their putative ancestors in *Nicotiana* (Greenleaf, 1941), in *Gossypium* (Harland, 1940) and in *Triticum* (Thompson, Britten and Harding, 1943). Recently *Brassica juncea* was artificially synthesised and its origin was traced with the help of cytological and cytogenetical evidences (Ramanujam and Srinivasachar, 1943). According to Morinaga (1934) *Brassica juncea* ( $2n = 36$ ) is an allotetraploid composed of the genomes of *Brassica campestris* ( $n = 10$ ) and *Brassica nigra* ( $n = 8$ ).

Two evidences were adduced to the allotetraploid origin of *Brassica juncea*. Firstly in a cross between *Brassica juncea* ( $n = 18$ ) and *Raphanus sativus* ( $n = 9$ ) there was complete absence of pairing among the *B. juncea* chromosomes themselves in the  $F_1$  hybrid. Secondly, when crosses were made on the one hand between *B. juncea* and *B. campestris* and on the other between *B. juncea* and *B. nigra* the Drosera scheme of pairing was observed. That is in the  $F_1$  hybrids (*B. juncea*  $\times$  *B. campestris*) and (*B. juncea*  $\times$  *B. nigra*) the configuration of 10 bivalents and 8 univalents and 8 bivalents and 10 univalents occurred respectively. In *B. juncea*  $\times$  *B. campestris* 10 chromosomes of *B. campestris* paired with 10 of *B. juncea*, leaving the 8 chromosomes of *B. juncea* as univalents.

It is clear from the regular formation of bivalents in these hybrids that the haploid set of *B. juncea* chromosomes is equivalent to the haploid set of the two species, *B. nigra* and *B. campestris* and that by doubling the chromosomes of the  $F_1$  hybrid got between them, plants resembling *B. juncea* could be produced.

Recently such an origin of *Brassica juncea* as an allotetraploid from *B. campestris* and *B. nigra* parents has been confirmed by the more direct evidence of synthesising the species by successfully effecting crosses between the two parents and subsequently inducing amphidiploidy by the application of colchicine (Ramanujam and Srinivasachar, 1943). Additional confirmation was obtained from the fact that there was uniform pairing between synthetic *B. juncea* and natural *B. juncea* when they were crossed. They crossed *B. campestris* and *B. nigra* and the  $F_1$  hybrid that resulted out of this cross possessed  $2n = 18$  and these appeared as bivalents and univalents during meiosis. Occasional cases of quadrivalent formation were also met

with Anaphase I and II were characterised by bridge formation. Fruit setting was very poor and only a few seeds could be available. The first generation of Amphidiploid was produced as a chimeral branch on  $F_1$  hybrid of the above cross. Two branches were treated with 4% colchicine in 50% glycerine. The branches treated showed fertility and an  $A_2$  generation of plants were raised from the seeds available in the fruits of the treated branches. The  $A_2$  generation possessed diploid set of 36 chromosomes and resembled in all respects those belonging to the species *B. juncea*. This amphidiploid crossed easily with the natural *B. juncea*. Pairing was complete thereby indicating that the haploid set of the amphidiploid was homologous to the haploid set of *B. juncea*. Indirect evidences as adduced by Morinaga (1934) stand confirmed by this direct evidence through artificial synthesis.

In a manner similar to the above mentioned cases, hybridisation and artificial induction of amphidiploidy led to the establishment of a true breeding species of *Sesamum* in this laboratory. The amphidiploid, details of whose characters are given in another paper, proved to be a stable true-breeding type and deserves an independent place along with the parental species, namely, *Sesamum orientale* and *Sesamum prostratum*. *Sesamum orientale* ( $2n = 26$ ) and *Sesamum prostratum* ( $2n = 32$ ) were crossed reciprocally. This resulted in a hybrid having  $2n = 29$ . Meiosis was found to be irregular because neither complete autosyndesis nor complete allosyndesis was observed. The result was that the hybrid proved to be sterile. A duplication of the chromosomes means the duplication of the parental chromosome sets. Then during meiosis there would be autosyndesis which would result in regular meiosis. Thus with this object in view the sterile hybrid was treated with colchicine and amphidiploidy was successfully induced.

Colchicine solution in tap water of strength 4% was applied in the form of drops at the terminal bud of young seedlings of the hybrid. The treatment was given twice a day on three alternate days. A cotton wool was placed at the region of application to prevent excessive evaporation of the chemical. The colchicine effect was revealed in the hybrid by its stunted growth and deformed leaves. Flowers were formed which were almost twice as big as those of the sterile hybrids. Viable pollen grains were formed and the treated seedlings yielded fruits with good seeds. Thus fertility was induced through amphidiploidy.

The cause of the fertility may be inferred as follows. The 29 chromosomes of the hybrid plant would have been doubled to 58 by the action of colchicine so that the somatic complement instead of having 16 chromosomes

of *prostratum* and 13 chromosomes of *orientale* would have 32 chromosomes of *prostratum* and 26 chromosomes of *orientale*. During meiosis no irregularity was noticed because the 32 chromosomes of *Sesamum prostratum* paired autosyndetically, to form 16 bivalents and the 26 chromosomes of *Sesamum orientale* parent paired auto-syndetically among themselves to form 13 bivalents, thus resulting in autosyndesis or intraspecific pairing in both the parents. The 29 bivalents observed in meiosis of the amphidiploid must be the total number of bivalents of both the parents. This amphidiploid has been established as a stable true breeding type evolving out of interspecific hybridisation followed by amphidiploidy.

The true breeding fertile hybrid resembles the *prostratum* parent more than the *orientale* including the perennial habit. Even the sterile hybrid shows greater resemblance to *Sesamum prostratum*. The cytological explanation for this may lie in the fact that both in the sterile and fertile hybrids there is a greater number of *prostratum* chromosomes. The  $F_1$  hybrid is only an annual whereas the amphidiploid is a perennial. Possibly the presence of a very large number of *prostratum* chromosomes, 32 in a complement of 58, is responsible for incorporating this parental feature also in the amphidiploid. Thus cytological investigations of many of the existing species, wild and cultivated, may well show that, in speciation, amphidiploidy has played an important role. Many of the existing forms may be proved to be amphidiploids provided their parental ancestors are discovered. Thus in evolution of new species, allopolyploidy has played an important part.

(d) *The possible origin of the cultivated Til, Sesamum orientale* Linn

From the cytological data gathered through interspecific hybridisation, it is safe to infer, in an empirical way, the possible origin of the cultivated Til. If, in the interspecific hybrid between *Sesamum orientale* Linn and *Sesamum prostratum* Retz, there was exhibited Drosera scheme of pairing, then it would have meant that the genome of *Sesamum prostratum* contained within it the genome of *Sesamum orientale* and consequently the two would be related ancestrally. But that is ruled out.

The frequent occurrence of 8 bivalents can be regarded as autosyndetic pairing amongst the 16 *prostratum* chromosomes, leaving the 13 chromosomes of *orientale* unpaired.

Or it may be that the 8 chromosomes of *prostratum* have paired with 8 chromosomes of *orientale* to form the bivalents. In this case 8 chromosomes of *prostratum* and 5 chromosomes of *orientale* are left out unpaired. This means allosyndetic pairing between 8 chromosomes of *prostratum* with 8 of *orientale*.

If it was allosyndetic, then all the 13 chromosomes of *orientale* must have paired with 13 *prostratum* chromosomes (Drosera scheme). It cannot be that 8 alone of *orientale* chromosomes could be homologous with 8 chromosomes of *prostratum* and the rest did not show any homology. So it is likely that the 8 bivalents frequently met with are the result of autosyndetic pairing among the *prostratum* chromosomes. This means that there is no pairing at all between *prostratum* and *orientale* chromosomes.

From the above two suggestions, two things are evident. (1) That the haploid sets of *orientale* chromosomes are not sufficiently homologous with one another to pair among themselves. So Til might have arisen through allopolyploidy. (2) An absence of pairing between the two parental chromosomes sets indicates the lack of homology between the chromosomes of *prostratum* and *orientale* and hence they could not have had a common origin.

This absence of homology as indicated by absence of autosyndesis amongst the *orientale* chromosomes must be due to either of two causes: (1) That the basic number of Til is 13 and that polyploidy has not played a part in the evolution and that the 13 gametic chromosomes, even though they may not show wide disparity in their morphology, are none the less structurally different from one another which results in their non-pairing. (2) The second alternative is that it should have arisen from a lower chromosome-numbered ancestor through the operation of polyploidy. If so, the absence of autosyndesis indicates that allopolyploidy has been the operating factor. Of these two alternative possibilities the latter seems the more likely.

In the previous investigation as well as in the present, the phenomenon of secondary association has been frequently met with and on the basis of maximum association, it has been suggested that 7 is the basic number of the species—meaning thereby that Til must have arisen from an ancestral form with a set of 7 haploid chromosomes. How this 26 chromosomed Til could have been evolved from such a basic number, through the operation of allopolyploidy, can be explained as follows:—

Supposing there were 2 ancestral species  $P_1$  and  $P_2$  each having 7 haploid chromosomes, one parent from  $P_1$  would have gametic genome A, B, C, D, E, F and G, whereas the other parental form  $P_2$ , possibly arisen through gene mutations, not involving numerical change, would have a genome of the same number of chromosomes (7),  $A_1, B_1, C_1, D_1, E_1, F_1$  and  $G_1$ . Then the parents are:

$P_1$	A		$P_2$	$A_1$	} Gametic genomes $n = 7$
	B			$B_1$	
	C			$C_1$	
	D			$D_1$	
	E			$E_1$	
	F			$F_1$	
	G			$G_1$	

A natural cross between the two forms  $P_1$  and  $P_2$  would result in a hybrid  $P_3$  having

$P_3$	A	$A_1$	} $2n = 14$ Sterile hybrid
	B	$B_1$	
	C	$C_1$	
	D	$D_1$	
	E	$E_1$	
	F	$F_1$	
	G	$G_1$	

The hybrid  $P_3$  is presumably sterile because chromosomes A and  $A_1$  from parents  $P_1$  and  $P_2$  are not homologous and so do not pair

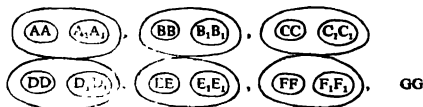
Somatic doubling takes place by some means, say, fusion of unreduced gametes. Then the chromosome sets in  $P_3$  will be doubled resulting in  $P_4$  having  $2n = 28$

$P_4$	$AAA_1A_1$
	$BBB_1B_1$
	$CCC_1C_1$
	$DDD_1D_1$
	$EEE_1E_1$
	$FFF_1F_1$
	$GG(G_1G_1)$ Deletion

Deletion of one pair of duplicated chromosomes in  $P_4$  results in the disappearance of one of the chromosome pairs say ( $G_1G_1$ ). Now  $P_5$  resulting after the deletion of one pair would contain  $2n = 26$  only ( $= S. orientale$ )

$P_5$	AA	$A_1A_1$	} $2n = 26$
	BB	$B_1B_1$	
	CC	$C_1C_1$	
	DD	$D_1D_1$	
	EE	$E_1E_1$	
	FF	$F_1F_1$	
	GG	—	

*Meiosis* in  $P_5$ —13 pairs are formed in 7 groups. Because of distant homology of A and  $A_1$  chromosomes AA bivalents is secondarily associated with  $A_1A_1$ . Thus we get 6 groups of secondarily associated bivalents, viz.,



GG remains unassociated,  $G_1G_1$  having been deleted during separation.

During the disjunction in  $P_2$

A	is separated from	A	$A_1$	is separated from	$A_1$
B	"	B	$B_1$	"	$B_1$
C	"	C	$C_1$	"	$C_1$
D	"	D	$D_1$	"	$D_1$
E	"	E	$E_1$	"	$E_1$
F	"	F	$F_1$	"	$F_1$
G	"	G			

So the gametic genome consists of:

A and  $A_1$ , B and  $B_1$ , C and  $C_1$ , D and  $D_1$ , E and  $E_1$ , F and  $F_1$ , & G. Since A and  $A_1$ , B and  $B_1$ , C and  $C_1$ , D and  $D_1$ , E and  $E_1$ , F and  $F_1$  are not homologous enough to pair, as evidenced in the non-pairing in  $P_2$  leading to its sterility, they do not show autosyndesis in the meiosis of the sterile hybrid ( $2n. 29$ )—*Sesamum orientale* (13)  $\times$  *Sesamum prostratum* (16).

The fact that these remained unpaired without any autosyndesis therefore implies an allopolyploid origin of Til in the manner suggested above.

So far no species of *Sesamum* having  $n: 7$  has been recorded. It is reasonable to expect such wild forms to be putative ancestors to the cultivated form. Only an extensive search for the wild ancestors of cultivated forms, such as organised by the Soviet, can throw light upon the problem.

(e) *The possible origin of Sesamum prostratum* Retz.

It is interesting to note that while one set of parental chromosomes (*Sesamum orientale*) do not pair among themselves, the other set of parental chromosomes (*Sesamum prostratum*) pair autosyndetically. The cause for the non-pairing among the *orientale* chromosomes is likely to be its origin as an allopolyploid in the manner previously described.

On similar lines, it may be supposed that autosyndetic pairing of *Sesamum prostratum* chromosomes in the sterile hybrid might be due to its origin as an autopolyploid from an ancestral form having haploid chromosome number

8 If that be the case then the ancestral form will have the somatic constitution as

AA	}	2n 16
BB		
CC		
DD		
EE		
FF		
GG		
HH		

Supposing doubling of chromosomes takes place then the zygote will contain 2n 32

AAAA	}	2n 32
BBBB		
CCCC		
DDDD		
EEEE		
FFFF		
GGGG		
HHHH		

Presumably no quadrivalent formation takes place and 16 bivalents are formed during the meiosis in *Sesamum prostratum*

Since A and A are homologous they pair and hence when they are in a new surrounding, namely, with *orientale* chromosomes in the sterile hybrid, they pair among themselves. They do not pair with *orientale* chromosomes because they are structurally different from them. This means that *Sesamum orientale* and *Sesamum prostratum* could not have arisen from a common ancestor. For, if that were so, the *Drosera* scheme of pairing would have been exhibited in the hybrid meiosis. If the frequent occurrence of 8 bivalents could mean autosyndetic pairing of 16 chromosomes of *prostratum* chromosomes, then it is likely that *Sesamum prostratum* has arisen as an autotetraploid from a parent having 8 haploid chromosomes in the manner described above.

In the meiosis of *Sesamum prostratum* there has been observed a regular absence of multivalents. Normally in autotetraploids, any four chromosomes ordinarily tend to form a quadrivalent group in meiosis. Often the synaptic association is such as to group the four members into two bivalents. Thus tetraploid sporocytes may sometimes exhibit the diploid number of bivalents "the double diploid" (Sharp, 1934).

According to Crane and Lawrence (1934) it seems that competition in pairing at prophase meiosis in an autotetraploid may give rise to univalent chromosomes instead of multivalents.

Autotetraploids may change the pairing habit of their chromosomes and the number of chiasmata may be reduced to one for each chromosome so that no quadrivalent formation can be formed (Darlington, 1939). In *Tulipa* tetraploids this is found to happen to a varying degree (Margaret Upcott, 1939). It is observed that the chiasma frequency of the tetraploids is low, are sexually reproducing, and have been subjected to selection because of their origin from diploid ancestors. They have been selected for fertility and hence the absence of multivalents.

Many species have been found to include a series of polyploid forms. In some cases these are indistinguishable from one another except by distribution. It is plausible to assume that these forms have arisen as autopolyploids with free pairing amongst their homologous chromosomes. This condition is still found in certain forms which have presumably remained unaltered since their origin.

However, in most cases of autotetraploids low fertility or complete sterility has been the rule. This is due to the irregular meiosis. Formation of multivalents is very common and their disjunction is unequal. Hence polymorphic grains are formed which are non-viable. So in speciation, autopolyploidy has not played as important a part as allopolyploidy. There are however cases where autotetraploids have established themselves as stable species. They show regular meiosis and bivalents have been found instead of multivalents. Upcott (1939) has recorded tetraploids showing no multivalent formation in *Tulipa*-species. The autopolyploids of *Tradescantia* (Anderson and Sax, 1936) is another instance in point. The above authors have reported the occurrence of an entire group of vigorous autopolyploids in the genus *Tradescantia*. These unlike the usual autopolyploids were found to reproduce themselves by seeds.

*Sesamum prostratum* may well be included under such autotetraploids. The perennial habit, the luxuriant growth and the high yield mingled with the non-susceptibility to any disease, either fungal or insect, may be an additional advantage acquired by *Sesamum prostratum* through autotetraploidy. According to Erlanson (1938) polyploid forms are better fitted to withstand Arctic or Alpine conditions while the diploids will simply perish. Navaschin (1929) has pointed out that "through changes in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions, and as a consequence, penetrate into new territory". Hagerup (1933) also has stated that "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid forms will not thrive".

If *prostratum* could have arisen through autopolyploidy and established itself as a stable form, then the presence of other wild forms like *Sesamum radiatum* Shum and Thonn ( $n = 32$ ), *Sesamum laciniatum* Klein ( $n = 14$ ) may be explained as a series of polyploid forms arising out of the putative ancestor having  $n = 8$ . Then *Sesamum radiatum* ( $n = 32$ ) will be an octoploid whereas *Sesamum laciniatum* ( $n = 14$ ) will be a tetraploid, having lost a pair of chromosomes in its meiotic complement (from  $n = 16$  to  $14$ ). Morphological evidences also may add proof to the inclusion of these two wild forms in the scale of polyploidy. *Sesamum laciniatum* and *Sesamum radiatum* have been found to grow luxuriantly maintaining at the same time the perennial habit. So, it might be that these forms, along with *Sesamum prostratum*, have arisen from an ancestor having a basic number of chromosomes  $n = 8$ , as autopolyploids.

#### V SUMMARY

Interspecific crosses between *Sesamum orientale* Linn and *Sesamum prostratum* Retz were effected reciprocally and the sterile hybrid was made fertile by artificial induction of amphidiploidy through colchicine. The cytology of the parents and the hybrids was studied in detail.

Details of meiosis of *Sesamum orientale*, one of the parents employed have been worked out. The peculiar persistence of the nucleus and its movements during the meiotic cycle are recorded. The other parent *Sesamum prostratum* has also been cytologically studied.

The irregular meiosis of the sterile hybrid and the occurrence of scattered bivalents and univalents in the metaphase plate, leading to the ultimate formation of abnormal sporads have been described fully.

The regular meiosis of the fertile amphidiploid is compared with the irregular meiosis of the sterile hybrid and the cause of this regularity is explained.

The nucleolus with behaviour of the spical regard to its persistence and movements is discussed.

Interspecific hybridisation as a guide to ancestral homology and the artificial synthesis of a new species are discussed in the light of cytological data gathered in the present investigation.

The origin of the cultivated T11 *Sesamum orientale* Linn from a putative ancestor having haploid number 7 through allopolyploidy is traced with the help of cytological details obtained in the hybrid meiosis.

The origin of the wild *Sesamum prostratum* Retz is also traced to an ancestral form possessing haploid number of 8 chromosomes through autopolyploidy.

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## INDEX TO VOL. XXVI (B)

### AUTHORS' INDEX

- Asthana, R. P. .. Studies on sclerotium-forming fungi, I, II, III, 93, 108, 117.
- Balakrishnan, M. S. .. *Phytophthora palmivora* Butler causing a seedling blight of *Hibiscus esculentus* L., 142.  
See Thomas and others.
- Gopalakrishna, A. .. Studies on the embryology of *microchroptera*, I, 219.
- Jacob, P. K., and Menon, M. Devidas .. Copepods of the West Hill Sea, 177.
- Kamath, Miss H. Sunanda .. The life-history of *Puccinia ruelliae* (B & Br) on *Ruellia prostrata* Poir., 1.  
Embryogeny of *Isotoma longiflora* Presl., 164.
- Kausik, S. B., and Subramanyam, K. Khan, A. K. M., and Singh, Inderjit .. The effect of the interaction between ions, drugs and electrical stimulation, as indicated by the contraction of human unstriated muscle, 205.
- Khanna, K. L., and Sharma, S. L. .. Studies in the anatomy of sugarcane stalk, I, 13.
- Koshy, T. K. .. The tapioca plant and methods for evolving improved strains for cultivation, 32.
- Krishnamurthy, K. V. .. See Raghavan and Krishnamurthy.
- Menon, M. Devidas .. See Jacob and Menon.
- Nayar, K. Karunakaran .. Undescribed males of two species of gall midges, 233.
- Nayar, S. Gopalan .. The newly hatched larva of *Periclimenes (ancylocaris) brevicarpalis* (Schenkel), 168.
- Padmanabhan, S. Y. .. *Fusarium* sp. parasitic on epiphyrops, a lepidopterous parasite of the sugarcane pyrrilla, 77.
- Raghavan, T. S., and Krishnamurthy, K. V. .. Cytogenetical studies in sesamum, I, 236.
- Ramakrishnan, K. .. See Ramakrishnan and Ramakrishnan.
- Ramakrishnan, T. S., .. The natural occurrence of ergot in South India, III, 136.  
.. See Thomas and others.
- Ramakrishnan, T. S., and Ramakrishnan, K. .. Additions to fungi of Madras, III, 7.  
.. A new rust on *Dalbergia paniculata* Roxb, 60.  
.. Revision of a rust on *Oldenlandia* spp., 64.
- Rangappa, K. S. .. Studies on the refractive index of milk, 125.

- Sarojini, Miss T. S., and  
Yogeswari, Miss L.      Aeration affecting growth and sporulation of some  
soil *Fusaria* in liquid cultures, 69.
- Sharma, S. L.      ..      See Khanna and Sharma.
- Singh, Inderjit      .      See Khan and Singh.
- Singh, Inderjit, and Singh,      The action of direct current on unstriated muscle, 211.  
Mrs. Sunita Inderjit
- Singh, Mrs. Sunita Inderjit      See Singh and Singh.
- Soumini, C. K.      ..      See Thomas and others.
- Subramanyam, K.      .      See Kausik and Subramanyam.
- Thomas, K. M., Rama-      Studies in the genus *Phytophthora*, I, 147.  
krishnan, T. S., Soumini,  
C. K., and Balakrishnan,  
M. S.
- Tiwari, K. K.      ..      Some stages in the development of the pineal complex  
of *Calotes versicolor* (Daud.), 195.
- Yogeswari, Miss L.      ..      See Sarojini and Yogeswari.

## TITLE INDEX

- Aeration affecting growth and sporulation of some soil *fusaria* in liquid cultures (Sarojini and Yogeswari), 69.
- Calotes versicolor* (Daud.), pineal complex, development, some stages (Tiwari), 195.
- Copepods of the West Hill Sea (Jacob and Menon), 177.
- Dalbergia paniculata* Roxb., a new rust on (Ramakrishnan and Ramakrishnan), 60.
- Ergot, the natural occurrence, in South India, III (Ramakrishnan), 136.
- Fungi of Madras, additions, III (Ramakrishnan and Ramakrishnan), 7.
- Fungi, sclerotium-forming, I, II, III (Asthana), 93, 108, 117.
- Fusarium* sp. parasitic on *epipyrops*, a lepidopterous parasite of the sugarcane *pyrilla* (Padmanabhan), 77.
- Gall midges, two species, undescribed males (Nayar), 233.
- Isotoma longiflora* Presl., embryogeny (Kausik and Subramanyam), 164.
- Microchiroptera, studies on the embryology, I (Gopalakrishna), 219.
- Milk, refractive index, studies, II (Rangappa), 125.
- Muscle, unstriated, human, contraction, the effect of the interaction between ions, drugs and electrical stimulation, as indicated by (Khan and Singh), 205.
- Muscle, unstriated, the action of direct current (Singh and Mrs. Sunita Inderjit Singh), 211.
- Oldenlandia* spp., a rust on, revision (Ramakrishnan and Ramakrishnan), 64.
- Periclimenes (ancylocaris) brevicarpalis* (Schenkel), the newly hatched larva (Nayar), 168.
- Phytophthora, genus, studies, I (Thomas and others), 147.
- Phytophthora palmivora* Butler causing a seedling blight of *Hibiscus esculentus* L. (Balakrishnan), 142.
- Puccinia ruelliae* (B & Br) on *Ruellia prostrata* Poir., the life-history (Sunanda Kamath), 1.
- Sesamum, cytogenetical studies, I (Raghavan and Krishnamurthy), 236.
- Sugarcane stalk, studies in the anatomy, I (Khanna and Sharma), 13.
- Tapioca plant and methods for evolving improved strains for cultivation (Koshy), 32.



